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LABORATORY DIRECTIONS

FOR

ELEMENTARY BOTANY

JAMES B. POLLOCK, Sc.D.
Associate Professor of Botany
University of Michigan

SECOND EDITION

GEORGE WAHR, Publisher
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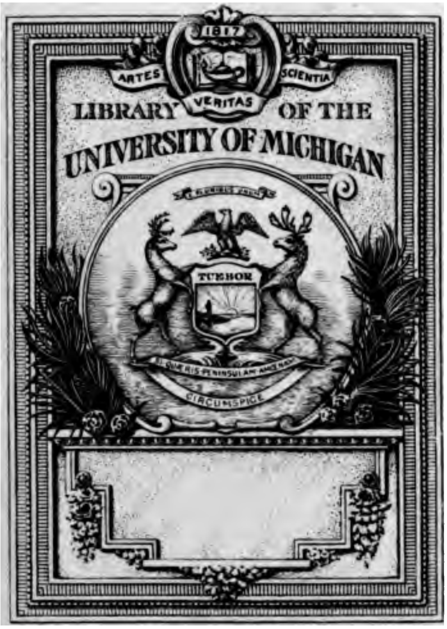
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LABORATORY DIRECTIONS

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ELEMENTARY BOTANY

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JAMES B. POLLOCK, Sc.D.
ASSOCIATE PROFESSOR OF BOTANY
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GEORGE WAHR, PUBLISHER
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PREFACE

This manual is the product of twenty five years' experience in teaching elementary botany to students in the University of Michigan. At the beginning of that period the author started with a similar manual used by his predecessors and associates, the late Professor Volney M. Spalding, of revered memory, and Professor Frederick C. Newcombe.

The outline of work has undergone considerable change since the time when the writer was given charge of the elementary course in botany at this institution. For these changes he has been largely responsible. As the students increased in numbers other men have coöperated in giving the laboratory work and lectures, and in the most recent years numerous discussions have been carried on as to how the course could be improved. Among those taking part in these discussions have been H. H. Bartlett, R. M. Holman, B. M. Davis, W. W. Tupper and others.

The work outlined is essentially that now given at this University, but the present edition contains some material not previously included in the manual as used here, and for this as well as for the arrangement and composition, the author assumes sole responsibility. It is hoped that in its present form it will be especially useful to the instructors in botany in the junior colleges associated with city high schools. In recognition of the fact that in most cases those instructors will have limited library facilities, material has been incorporated into the present work that might properly be reserved for a text book rather than a laboratory manual. The reason for so doing is that some of the work herein presented is so new that it has not yet found a place in any textbook of botany. This is the justification for the paragraphs on vitamins and the newer knowledge of nutrition.

Other topics are presented so inadequately in most textbooks that it has seemed worth while to give a new presentation. For this reason the carbon and nitrogen cycles have been included.

Whether the results justify the attempt at a new presentation by the writer, may be left to the judgment of the experts in the field concerned and of the teachers who may find the manual adapted to their needs.

At the University of Michigan the elementary course in botany is given for one semester, followed or preceded by a semester of Zoology. The two give credit to the students as general biology, 4 credit hours each semester. After the present date each student will have one lecture, two quizzes, and 6 hours of laboratory work per week. Previously he had two lectures and one quiz with the same amount of laboratory work. The time given by the student, counting that spent in both study and recitation, is counted as equal to a study in which there are four recitations per week without laboratory work.

Among the changes in this edition as compared with earlier ones are:

1. The students are given more information directly, enabling them to understand more quickly the facts they are really expected to work out for themselves. In any case much of this must be done for each student, either by the instructor orally or by means of printed directions. With small classes the former is probably more advantageous. With large classes the latter seems necessary.

2. Experiments in plant physiology have been added. It is expected that these will be done almost wholly by demonstration. Lack of laboratory space, time, apparatus, and teaching force make it impossible to do this as individual work for each student.

The advantage of experiments is that they give the student an opportunity to see as a unit all phases of the scientific method as used in research, of which a noted educator has said that it is the only method by which progress is made in any subject. It is especially important that the student should see clearly the difference between observed results and conclusions drawn from them, and the necessary logical relation of the latter to the former. It is believed that these experiments, to a more adequate degree than any other one phase of the work, will give to students the "discipline of science," which is a training in the scientific method of work, in particular as it is applied to the field of plant life.

3. It was agreed by the men coöperating in the teaching

of elementary botany in the University of Michigan, that students should be asked to learn the Divisions and Classes of the plant Kingdom and their characteristics. To facilitate this, the author has given a characterization of each Division and Class for which material is presented for study. He has attempted to give a few of the most distinctive characters, without too much technical material and also to indicate briefly the general biological significance or the points of contact with human life. No attempt has been made to teach applied botany specifically, but in the discussion of some topics it is pointed out how the principles of plant life underlie certain applications to practical affairs.

4. In certain parts, the work has been made less intensive and more extensive. Formerly a few forms were studied in great detail. That custom has been changed gradually, and in this edition, specifically in the study of the gymnosperms, a Key is provided for identification and brief description of as many species as time and material allow. The Key was prepared with the material in mind that is generally available in Michigan. It is recommended also that laboratory or field studies be made of the commoner angiosperms, though no special Key is provided for them.

There is more work outlined than can be completed in one semester. This leaves room for a choice of material by the teacher, in those institutions in which elementary botany is given for one semester only. The selection may be determined by the teacher's interest or the material available. In the University it is shortened by leaving out some of the algae and fungi and omitting parts of the study of liverworts, mosses, and ferns. It is not necessary to study in detail the life history of both a gymnosperm and an angiosperm. An external study of several of the former and a detailed study of the latter are recommended. A year's work may easily be provided by adding systematic botany of the angiosperms.

JAMES B. POLLOCK.

Jan. 2, 1922.

LABORATORY DIRECTIONS
FOR
ELEMENTARY BOTANY

THE PLANT CELL

All plants are composed of cells, which are sometimes called units of structure. The material to be studied first is an aquatic flowering and seed-bearing plant, whose leaves are suitable for the study of the cell. This plant is Elodea canadensis.

A. Note that the Plant grows submerged in water. It is therefore aquatic. As in seed plants generally, it has **stems, roots,** and **leaves**; but is able to grow in jars in the laboratory without roots. It therefore must absorb all the materials necessary for its life from the surrounding water and through the surfaces of all its organs. Note the arrangment of leaves on the stem. Are they in whorls, that is, circles of several at the same level? Are they opposite, that is, two at the same level on opposite sides of the stem, or are they alternate, that is, in a spiral arrangement?

B. **The Leaf.** Remove with the forceps a fresh leaf from a growing tip and mount in water. Is the leaf petiolate (having a stalk), or is it sessile, (attached directly to the stem by a broad base)? What is its size, expressed as the greatest length and width? What is its shape? Examine it with the low power of the compound microscope. What is the character of the margin? Notice a dark line through the middle of the leaf from the base to the apex. This is the mid-rib· The broad expanded portion is the blade, or lamina. Dark lines running parallel to the mid-rib are not secondary ribs, but intercellular spaces, filled with some gas, which in bright sunlight is likely to be pure oxygen. Why?

Looking at the finer structure of the leaf, note the boundaries of individual cells, showing as very thin white lines. Examine all parts of the leaf and note differences in size, shape, and color of cells. If the diameter of the field of your microscope has been measured, you can easily estimate the size of objects quite accurately. What is the actual size of the cells?

Draw in outline the leaf as a whole and draw on a larger scale a portion showing the relative size and arrangement of cells in outline.

Before going farther write in your notebook a description of the plant and the leaf as you have studied it.

C. **Individual Cells.** With the high power study individual cells.

1. Note first the **cell wall,** its color, its position, its thickness estimated as a fraction of the short diameter of the cell. This wall is composed of **cellulose,** whose chemical formula is approximately $n(C_6 H_{10} O_5)$, the value of **n** being unknown. The wall is the most rigid part of the cell, and helps to give rigidity to the plant as a whole. Does each cell have its own separate wall or are the walls common to two adjacent cells?

2. Observe in some cells a substance in motion carrying green granules, the **chlorophyll bodies** or **chloroplasts,** around in the cell. Choose a cell in which the chloroplasts follow each other in single file along the wall, with some distance between them. Note the continuously flowing, granular, very transparent substance. This is the **cytoplasm.** Does it carry the chloroplasts along or do they move the cytoplasm? Give a reason for your answer. How thick is the layer of cytoplasm and chloroplasts estimated as a fraction of the cell diameter?

3. **The nucleus.** Somewhere in the cell, lying imbedded in the cytoplasm, is a rounded body, much larger than the chloroplasts. It is nearly transparent, but is generally more dense than the cytoplasm. It is the nucleus, and usually a denser body, the nucleolus, may be seen within it. What shape is the nucleus? What is its diameter as compared with that of the cell? Where is it located?

4. **The vacuole.** The space within the cell, completely surrounded by the layer of cytoplasm and chloroplasts, is called the vacuole. It is filled with a watery solution called cell sap, which is composed of water and various substances dissolved in it. Sugar is one of the most common substances in the cell sap, but many others may be present, as, inorganic salts, organic acids, and their salts, etc. What is the shape of the vacuole? What proportion of the cell volume does it occupy?

5. **Chloroplasts.** Study the chloroplasts more in detail. What is their size? Of what shapes are they? Note the shape when lying quietly along the top wall of the cell, and also when in motion along the lateral walls. If these are views from different sides of the chloroplasts, what is their actual solid form? As the chloroplasts move about the cell, note the rate of motion. Is it in the same direction in all cells? Look for slender strands of cytoplasm flowing across the cell through the vacuole, usually from the vicinity of the nucleus. The cytoplasm, the nucleus, and the chloroplasts together are called protoplasm. The protoplasm alone is the living part of the cell. Neither the cellulose wall nor the cell sap filling the vacuole is alive. Protoplasm has been called the "physical basis of life", and is essentially similar in both plants and animals. This is a fundamental fact for the science of **biology.** Make two drawings of a single cell on a large scale, short diameter at least half an inch, one a view of the upper surface, the other a median, optical section, showing what would be cut by a plane passed through the middle of the cell longitudinally, midway between top and bottom.

6. **Test for Starch.** Mount a leaf of Elodea in an iodine solution, then examine under high power. What effect has iodine on the cytoplasm, the nucleus, the chloroplasts? Note the chloroplasts particularly. Cells near the base of the leaf and near the mid-rib are most likely to show results. Look for blue or blue-black granules within the chloroplasts. These are starch grains, as iodine turns starch blue or blue-black. How large are the starch grains compared to the chloroplasts? How many grains in one chloroplast? In sunlight chloroplasts make sugar and, if there is an excess of sugar, they change some of it to starch. Hence the starch was made, chemically synthesized, in the chloroplasts, out of sugar. Sketch a few chloroplasts containing starch granules.

The cell you have studied may be called a typical plant cell, since it has all the parts found in the green portions of plants. The leaf, or blade, of Elodea is only one cell thick at the margin, and two cells thick from margin to mid-rib. It is simpler in structure than most leaves, especially those of land plants, whose leaves are borne in the air and show a greater degree of complexity in structure.

LEAVES, THEIR SHAPES AND CELL STRUCTURE

On young plants of squash (Cucurbita maxima) or pumpkin (Cucurbita Pepo), notice the two kinds of leaves, seed-leaves or cotyledons and foliage leaves.

A. **Leaf arrangement.** Are the leaves opposite, whorled or alternate? Are they petiolate or sessile? Do they have stipules? These are small leaf-like structures at the base of the petiole.

B. **Description of leaf blade.** In describing the leaf blade use the following order:

(1) Simple or Compound;
(2) Method of veining;
(3) Size;
(4) Shape;
(5) Kind of margin;
(6) Kind of base;
(7) Kind of apex.

A leaf is simple when the blade is all one piece. A compound leaf has the blade divided into a number of separate leaflets, all connected to the stem by one petiole. Give size as range of length and breadth in centimeters. If the leaves are not full size, ask the instructor what size they attain.

The **types of veining** are **parallel veined** and **netted veined.** Parallel veins may run from base to apex of the leaf blade, or from the mid-rib to the margin. Netted veined leaves may be **pinnately** or **palmately veined.** The former have one chief mid-rib; the latter have several ribs or veins of nearly equal size, radiating from the base of the leaf. The leaf is divided into a network by the small veinlets.

The chief **shapes** of leaves are: **linear,** long and narrow with parallel sides; **lanceolate,** long and narrow but wider at the base and tapering gradually to the apex; **oval,** somewhat longer than wide and rounded equally at both ends; **ovate,** rounded at the base and more or less pointed at the apex; **orbicular,** or **rotund,** having a circular outline. If the leaf is broader above the middle, the prefix "**ob**" is used with some of the terms just defined, as, **obovate, oblanceolate, etc.**

Shapes that are intermediate between those named above may be described by combining two of the terms, as **ovate-lanceolate,** etc. These technical terms are a means of shortening descriptions, telling in one word what would otherwise require a long phrase.

The **margins** of leaves are **entire** (smooth), **serrate,** (with teeth pointing toward apex), **dentate** (teeth pointing straight outward), **crenate** (scalloped), **lobed, cut,** or **divided.**

Leaf **bases** are **pointed, rounded, heart-shaped, kidney-shaped, arrow-shaped,** etc.

The apex may be **taper pointed, acute, blunt, rounded,** or **indented.**

Using the above outline, describe both the cotyledons and foliage leaves of Cucurbita. Their differences are associated with partly different functions. The cotyledons are used for storage of food in the seed, for the use of the young plant; but they come above the ground and also serve for foliage. The foliage leaves are primarily for the manufacture of food, especially of sugar. They may also serve for temporary storage. You found starch in the leaves of **Elodea canadensis.** They also serve other purposes in certain plants.

Draw both the cotyledons and the foliage leaf, showing the characteristics made out, labeling all parts. Also draw and describe the leaves of any other plants given for study. Leaves and their characters are in part the means by which different species of plants are distinguished. You may easily learn to know the common plants, shrubs, trees, etc., by the shapes and other characters of their leaves.

If you are taken on field trips, collect specimens of the leaves of each species studied. Notice out of doors that for every species, and even for every individual, there is a **range of variation** for shapes and size of leaves. They are not exact duplicates of one another. This variation of parts is one of the fundamental facts of biology.

C. **Cell structure of the leaf.** 1.. **Epidermis** in surface view. Take fragments of epidermis stripped or sliced from the lower surface of some leaf, (Tradescantia, Bryophyllum, and the Boston fern are suitable) and study under low and high power. These fragments give you the surface view of the epidermis. Note the shape of the cells composing most of the epidermis and also here and there pairs of smaller cells with slit-like openings between them. These are the **stomata** and the two cells composing each **stoma** are called **guard cells.** The opening allows an exchange of gases between the outside and the interior of the leaf. Are the stomata open or closed? How wide is the opening compared with the width of the guard cells? How do the latter differ from the other epidermal cells in size, shape, and cell contents?

Draw a portion of the epidermis, showing the differences observed.

If the upper as well as the lower epidermis is studied, note the difference in the presence, or relative numbers, of stomata on the two sides of the leaf.

D. Take **cross sections** of leaves, mounted and stained, and comparing them with sections cut from fresh leaves, make out the following structures:

1. **Epidermis,** a layer of cells on both the upper and lower surfaces of the leaf. What is the shape of these epidermal cells? On the upper epidermis look for a thick **cuticle** on the outer portion of the outer wall. This cuticle is more or less impervious to gases, oxygen, carbon-dioxide, etc., and to water whether as liquid or water vapor. Look for the **stomata.** Do they occur in both upper and lower epidermis? How are the guard cells different in shape, size, and thickness of walls from the neighboring epidermal cells? Are there **trichomes,** or **hairs** present? Are they on one or on both sides of the leaf? If on one are they on the same side as the stomata or not? Ask the instructor their use.

2. The tissue within the epidermis consists of **mesophyll.** The portion of the mesophyll beneath the upper epidermis consists of one or two layers of compact oblong cells, the **palisade parenchyma.** The remainder of the mesophyll consists of looser and more irregular cells and is called the **spongy parenchyma.** Comparing with cross-section of fresh leaves, find which cells have chloroplasts and which have not. The palisade and spongy parenchyma together are sometimes called chlorenchyma. Why? Note also the presence of cytoplasm and nucleus. Note the intercullar spaces between the parenchyma cells, large and irregular in the spongy parenchyma, smaller and more regular in the palisade tissue. Do the stomata open into the cells or into the intercellular spaces?

3. At the middle of the leaf you will see a cross-section of the **mid-rib.** This consists of a **vascular bundle** at the center, more or less completely surrounded by thick-walled cells, which give much **mechanical strength** to the leaf. The ribs and veins serve two purposes: they furnish a mechanical framework to support the softer tissues of the leaf, and they contain the vascular bundles which are the conducting tissues, or lines of transportation. That portion of the bundle nearest the upper side of the leaf is called **xylem.** It forms the path along which water and mineral matters are carried from the stem to all parts of the leaf. That portion of the vascular bundle nearest the lower surface is called the **phloem.** It is the tissue that conducts the food made in the leaf to other parts where it is stored or used in growth.

Draw the whole section of the leaf in outline, and make an exact drawing showing cell structure in a strip from top to bottom of the leaf section.

PHYSIOLOGY OF LEAVES.

In describing physiological experiments, for each experiment tell first what was done and how it was done. Second, as a separate part of the record, give observed results. Third, discuss the significance of these results, comparing those of the experiment proper with the "control" or "check." In experiment 1, the lighted part of the leaf is the check against the part experimented on by shading it. The check is necessary for the logic of the conclusion. Fourth, draw whatever conclusions the facts warrant, but none that is not warranted by the observed results, or by these results taken with other known facts. In other words, see that there is a **logical connection** between your facts and your conclusions. The conclusion may have only a certain degree of probability, instead of a high degree of certainty. If this is the case, the fact should be recognized.

In the study of natural science, especially biology, the evidence does not always allow perfect proof of conclusions, such as is found in mathematical demonstrations.

Describe the experiments shown in the laboratory and the green-house, demonstrating the function of leaves.

1. A leaf of pumpkin was nearly all darkened by black paper for a period of three days. Only the basal lobes of the leaf and the part under a pattern cut in the black paper on the upper side were exposed to light during this period.

The leaf was then cut from the plant, dipped in boiling water for a few minutes, and placed in alcohol over night to remove the chlorophyll. It was then soaked in a solution of iodine in alcohol, and in a few minutes one portion of the leaf turned a blue-black. The rest of it remained nearly colorless. What is the material that stains dark? Is it in the lighted or the darkened portion? Why is it there and not in the other portion?

Draw two conclusions from the observed facts, as to the function of leaves and as to one of the necessary conditions for it.

This experiment also shows that material is either conducted away from the leaf or is used up. What facts shown allow this as a logical conclusion?

2. From the water plants in the aquaria in the green-house, bubbles of some gas are rapidly given off. Count the bubbles in a certain stream for half a minute, while the plants are in direct sunlight, and after shading the plant for a few moments, count them again. What is the difference? The bubbles are pure oxygen. Explain their origin and the difference in rate in the lighted and shaded conditions.

3. Amount of water contained in plants. Several leaves or whole plants are weighed fresh, then dried at 100°-110° C. until weight is no longer lost, then weighed again. The loss in weight is the amount of water in the fresh plant. Take the figures given you by the instructor and calculate the percentage of water present.

4. Several pumpkin plants or single leaves are to be weighed and then placed with the plant stems or leaf petioles in bottles of water, the water filling the bottles full to the neck. They are then set in the greenhouse for a day or more, and at intervals the bottles are filled as before, and the amount of water necessary to do this may be measured, or the loss may be obtained by weighing the bottle and plant at first and again before filling. The loss from the bottles with plants may be compared with that from bottles of the same size filled with water alone. These serve as checks. The difference between

the average loss from the bottles alone and the bottles with plants, gives the amount of water lost by the plants. This loss of water by plants is called **transpiration,** and takes place mostly through the leaves, because they expose more surface, and are provided with abundant stomata on at least one side, and in many leaves on both sides.

Your instructor will tell you the results of these transpiration experiments. Given the weight of the plant or leaf and of the water lost in a certain time, calculate the percentage lost in 24 hours on the basis of the original weight, and also calculate the total amount which would be lost during a growing season of three months, and its ratio to the weight of the plant. Notice the great amount of water required by plants, both that which they contain at one time, and that which passes through them and is lost by transpiration.

STEM STRUCTURE.

A. With plants of Cucurbita and Tradescantia or other species before you, note that stems are made of **nodes,** the joints or points where the leaves arise, and **internodes,** the intervals between the nodes. Notice where the branches of the stem take their origin. The angle between the leaf and the stem where branches grow is called the **axil** of the leaf. On the Cucurbita you will find **tendrils.** Do they have the position of leaves or branches? Sketch the curved tip of a free tendril. With a small, somewhat rough object, as a pencil, rub the tendril on the inner side of the curved tip. Look at it at intervals of one to several minutes and note the effect. What happens? How long does it take? Sketch again after the reaction. What happens to a tendril that has clasped a support? Of what use is this to the plant?

On the basis of stem structure there are two different kinds of stems: one, characteristic of those plants having two cotyledons, called **dicotyledons,** or **dicotyledonous plants;** the other, characteristic of plants with one cotyledon, called **monocotyledons,** or **monocotyledonous plants.**

B. **Zea mais,** or **Indian corn,** is one of the monocotyledons. Study cross sections of the stem for its structure. Make out the following parts:

1. **Epidermis,** the outer layer. How do the epidermal cells differ in size, shape, and thickness of walls from the majority of the cells in the section?

2. One or more **subepidermal layers** with thick walls, which, with the epidermis, contribute greatly to the mechanical strength of the stem. In a mature dry stem, notice the difference in mechanical strength of the outer and inner layer.

3. The **fundamental tissue** filling up most of the center of the stem, in which the vascular bundles are distributed. The cells forming the fundamental tissue are of the kind known as parenchyma. Note their size, shape, thin walls, and the thin layer of cytoplasm, if any is visible, in the sections. Note also the intercellular spaces where three or four cells are adjacent to each other. These intercellular spaces communicate with one another throughout the plant. In the leaf you saw that they open to the outside of the plant through the stomata. They are the air channels, or means by which gases in the air can get into the interior of the plant; and gases given off by the plant into the intercellular spaces get outside the plant.

4. The **fibro-vascular bundles.** These are numerous and scattered throughout the diameter of the stem. In a bundle which shows the structure clearly, note two different regions:

a. The **xylem** or wood region, marked usually by two very large and one or more smaller vessels, the chief path for the passage of water; and,

b. The **phloem,** the region which conducts food. The characteristic cells of the phloem are sieve tubes and companion cells, arranged in such a way that a small companion cell with granular contents is surrounded by four larger sieve tubes, which are nearly empty. Is the xylem or the phloem of the bundle nearer to the center of the stem? The bundle is usually surrounded by thick-walled cells forming the bundle sheath.

Draw a diagram of the whole section of the stem, showing the distribution of vascular bundles. Outline the section as a whole, outline each bundle, and the large vessels in the xylem. Draw in detail a single bundle, cell for cell, as accurately as you can do it. Label all the parts represented on each drawing. Draw several parenchyma cells.

c. In the longitudinal sections of corn, describe and draw pith cells; also the different kinds of vessels in the vascular bundle: **annular, or ring vessels; spiral vessels;** and **pitted vessels.**

C. **Ricinus communis,** the plant that produces castor oil in its seeds, is one of the dicotyledons. Study cross sections of the young stem when it shows the primary structure. From the outside note:

1. The **epidermis,** a single layer of cells. Notice the size, shape, and thickness of the walls of the cells that compose it.

2. The **cortex,** a rather broad zone of parenchyma cells, ending on the inner side with the endodermis, which is often filled with starch, and forms a sheath around the part called the stele, or central cylinder.

3. The **fibro-vascular bundles.** How many are there? How are they arranged? How does this differ from the arrangement in Indian corn? As in the corn there are both xylem and phloem present, the latter nearer the surface and the former nearer the center. The phloem is sometimes called the bast, and the outer part of the phloem is a group of thick-walled cells, long and slender like fibres, and called bast fibres. The presence of such fibres is the reason for calling the bundles fibro-vascular bundles. The bast fibres of flax are the structural elements which are spun and woven into linen. A tissue which is not present in the bundles of monocotyledonous plants is found in Ricinus and other dicotyledons. It is the **cambium.** Look for it between the xylem and the phloem of the bundles; it consists of very thin-walled cells, and it is the layer that retains the power of division, and therefore it can form new cells.

4. The **pith** lies at the center of the stem and is connected to the cortex by masses of cells called

5. **Medullary rays.** The medullary rays occupy the spaces between the bundles and connect pith to cortex. They as well as the pith and cortex are composed of parenchyma cells. All parts of the stem interior to the cortex are called the central cylinder or stele.

Draw a diagram of the whole section, showing all the parts studied, and draw accurately a wedge-shaped portion from epidermis to pith, showing one fibro-vascular bundle complete. Label all parts.

D. **Secondary thickening.** Some stems retain the primary structure throughout the life of the plant. Others increase in thickness by what is called secondary thickening.

In the cross-sections of older stems of Ricinus, notice that the **cambium** of the primary bundles has been connected across the medullary rays by a secondary cambium, the two forming a complete cylinder of cambium. This cambium undergoes cell division. Some of the cells formed differentiate into phloem cells on the outer side, but a larger number differentiate into xylem or wood on the inner side of the cambium. Also new bundles of xylem and phloem form between the primary ones, and these are secondary bundles.

Draw a wedge-shaped portion, showing two bundles with the secondary cambium.

E. **Aristolochia,** a perennial woody stem. In trees, shrubs, and climbing vines that live more than one year, the secondary thickening produces **annual rings** in the xylem, or wood, as well as changes in some other parts of the stem.

1. In sections of a younger stem of Aristolochia, note the primary structure, much like that of Ricinus but with certain differences, as the number of the bundles and the width of the medullary rays. Also instead of a bundle of bast fibres for each vascular bundle, there is a continuous zone of thick-walled, fibre-like cells, composing the outer portion of the central cylinder, which is called the pericycle. Make a diagram of this stem, outlining and labelling all parts.

2. Now compare with this the section of a stem two or more years old. What change, if any, has each region or tissue undergone, as to increase in thickness and change in character of cells? Note especially the thickness of the wood or xylem, relative to the diameter of the whole section. Does it show rings of growth? How many? How old is the stem therefore? Usually, but not invariably, there is one ring of wood for each year's growth.

3. On the outer margin of the section, you may notice portions of a layer of **cork.** It originates from another cambium called the **cork cambium.** Note the thin cell walls, the rectangular shape of the cells, and their arrangement in radial rows.

On stems that grow by secondary thickening, cork takes the place of cuticle and epidermis, and is, like the cuticle, impervious to water. Hence it protects the twigs and trunk of woody plants from loss of water through their surfaces.

Make a drawing of a wedge-shaped portion of the older section to show the structure of all the regions from cork to pith.

F. The **grain** of wood. Study blocks of wood in cross, longitudinal, and tangential sections. In each of these views distinguish the annual rings and the medullary rays. The particular way in which these are cut at the surface of the block or board is what gives the wood its characteristic "grain." Quarter-sawed oak is sawed so that the surface is parallel to the radius of the trunk of the tree, and the beautiful surface so much desired in furniture and wood finishing is due to the medullary rays. In order to get as much as possible of this surface, the log must be turned frequently in sawing, or quartered, hence "quarter-sawed oak." An expert may distinguish species of wood by the characteristic grain.

PHYSIOLOGY OF STEMS AND LEAVES.

Describe the experiments showing the functions of the stem and the reactions of stem and leaves to environment.

1. **Path of transpiration current.** Examine a plant which has been standing in a dye or coloring matter that the plant can absorb. Determine where the water carrying the dye passed up the stem. This can best be done by looking at cross sections of the stem and noting which tissues are colored by the dye. What tissue is the path for water?

2. Reactions of stems and leaves to stimuli. Plants adjust themselves to their environment by reacting to various forces in that environment, which act as stimuli. These reactions are exhibitions of that fundamental property of living protoplasm called **irritability.**

A. Six plants of one species are placed, three in the light and three in darkness. In each location one is hung upside down, one is laid with its stems horizontal and its leaves facing the horizontal direction, and one is set in the normal upright position. The plant in the normal position is the check for the others under otherwise similar conditions, and the three plants in the light serve as a check for those in darkness.

After one or more days note the difference in position of stems and leaves for each plant, as compared with the other under the same condition; and compare the set in the dark with that in the light. Draw whatever conclusions the facts allow you to draw as to the cause of the different results in the same situation and in the two situations.

One of the stimuli acting on plants in the dark is **gravitation.** The reaction to this stimulus is an exhibition of **geotropism.** An organ that turns away from the earth shows **negative geotropism**; one turning toward the earth shows **positive geotropism,** one taking a position at right angles to the vertical is said to show **dia-geotropism.** Name the kind of geotropism shown by stem, petiole, and leaf blade respectively in this experiment.

B. Plants are put in a cage of wood or black paper with an opening in one side, so they are lighted from only one direction. What reaction do the stems show? The leaves? What is the stimulus that causes this reaction? Does the stimulus in this case work together with gravitation or in opposition to it in determining the direction of stems and the position of the leaves? Explain this clearly. The reaction to the stimulus of light is called **phototropism,** or sometimes **heliotropism.** Here also there may be **positive, negative,** and **dia-phototropism,** depending upon whether the part of the plant reacting turns toward the source of light, away from it, or takes a position at right angles to the direction of the light ray. What kind of phototropism do stems and leaves show respectively? Do the petiole and blade of a leaf react alike?

C. **Reaction of the sensitive plant.** Notice a sensitive plant, **Mimosa pudica,** standing in the light, its twice-compound leaves with many small leaflets arranged in pairs.

When the plant is touched by the hand, what two reactions take place? Where is the bending in the petiole? At this point there is a special motor organ called the **pulvinus.** Each leaflet stalk also has a pulvinus.

Compare the position of the leaves of **Mimosa** in the light with another plant of the same kind that has been standing in darkness. Is the position of leaves and leaflets like that of the plant reacting to touch or not? Plants of the family Leguminosae, to which Mimosa belongs, generally have a night and day position for the leaves. The motion of the leaf and leaflets is brought about by a shortening of the cells on one side of the pulvinus. This shortening is produced by movement of water out of the cells into the adjacent intercellular spaces, as a result of the stimulus. The reaction might be said to be brought about by an **hydraulic process.**

In the evening as it is growing dark, notice the position of the leaflets of clover, beans, the locust tree, or other plant belonging to the family Leguminosae. How does it differ from the position occupied during the day? If you observe these same leaflets in the middle of a hot dry day, you may see a still different position.

D. **Leaf Mosaics.** By reactions, mainly to light, all the leaves on a plant so arrange themselves that they shade each other very little, and all get the best position possible for receiving light. Leaves adjusted in this way show what are called "leaf mosaics." Note the leaf mosaics on the English ivy or other plants.

Leaves which do not have a pulvinus lose their capacity to change their positions when they cease growth, and have what is called a "fixed light position," which is about that at which they get the best average illumination.

ROOTS.

Roots, like stems, have **epidermis, cortex,** and **central cylinder,** or **stele.** They also have vascular bundles, but the arrangement of xylem and phloem in the bundles is different.

Whereas in the stem each fibro-vascular bundle has xylem and phloem on the same radius, in the root, xylem and phloem alternate on different radii. Roots also differ from stems in having a root cap, and with the exception of the primary root, they have an endogenous origin, that is, they originate within the root or stem on which they grow, and break out through the surface.

A. Note the roots of **seedlings** of radish or other species **furnished,** with their **root hairs.** How long are the root hairs, compared with the root diameter? Notice how near the hairs grow to the tip of the root. What advantage are the hairs in the absorption of water and dissolved substances?

Draw a seedling showing the root and root hairs.

B. **Cross section of a root** of maize or iris showing primary structure. In the cross section furnished, make out three regions as follows:

1. **The epidermis,** a single layer of cells on the outside of the root. Are there any root hairs growing from the epidermis cells?

2. **The cortex.** Is its width proportionately greater or less than that of the corresponding region of the stem? The specialized inner layer of the cortex is the **endodermis.** It bounds

3. The **central cylinder,** also called the **stele.** In the latter distinguish:

a. The **pericycle,** a layer of cells adjoining the endodermis.

b. The **primary xylem** groups. How many of them are there? The wood that is first differentiated occurs at the outer point of each group. That which is subsequently differentiated extends inward toward the center of the cylinder and in many roots the center itself is xylem. Is such the case in the plant you are studying? If not, pith will be found at the center. How does the outer xylem differ from the inner? Xylem walls are stained a reddish color by the stain or dye used in preparing the sections.

c. The groups of **primary phloem.** These alternate with the xylem groups, and occur on different radii. How does the phloem differ from the xylem as regards the size of cells, the thickness of cell walls? The cells of the phloem are stained more bluish or violet in color.

4. Draw the cross section, outlining its parts and draw a wedge-shaped portion on a larger scale, showing all the tissues found in detail.

C. **Longitudinal section of young root.** In longitudinal sections of young roots the beginning of differentiation of cells may be studied. Select median sections.

1. At the root tip distinguish between the **root proper** and the **root cap.** At the tip of the root proper there is a group of cells known as the primary meristem, from which are derived by repeated cell division and differentiation, all the structures of the root and cap.

2. The **root cap** is a tissue of loose cells which is worn off at the outside as the root penetrates the soil, and is constantly renewed from the inside. It protects the growing region against mechanical injury. There is no corresponding structure at the apex of a stem? Why?

3. Close to the growing point of the root proper it is possible to see evidence of the differentiation which ultimately produces the various tissues that have been seen in the cross-section of a more mature root. Look for three regions, as follows:

a. The **dermatogen,** a single layer of cells giving rise to the **epidermis,** the external layer of the root proper;

b. The **periblem,** a broad zone of cells on both sides of the section, internal to the dermatogen, giving rise to the cortex;

c. The **plerome,** a cylinder of cells at the center giving rise to the central cylinder or stele in the mature root.

4. Make a diagram of the longitudinal section, showing all of the regions mentioned and draw a strip of cells across the root to show accurately the differences between the different kinds of cells.

Mitosis or Indirect Nuclear Division, and Cell Division.

Growing root tips provide favorable material for the study of mitotic nuclear division. Many cells are dividing, so that several stages of mitosis may often be observed in a single section. Using prepared slides of the root tip of the onion, find and draw cells with dividing nuclei, making out a sufficient number of stages to give you a clear idea of the process as a whole.

A. **Nuclear division.** Note the following stages with the understanding that a stage is not a stopping point, but a state of progress in a continuous process, which is completed in a few hours from the beginning:

1. **Resting nucleus.** This term is applied to nuclei which are not in the process of division. Notice the size of the nucleus; the nuclear membrane; a fine network of threads called **linin** extending throughout the nucleus; attached to this at various points deeply stained granules of **chromatin**; somewhere within the nucleus a large highly stained granule, the

nucleolus. The chromatin is composed of **nucleo-protein,** a protein which contains the element phosphorus. Draw each stage as you find it, but arrange drawings so that they are in the order of succession.

2. **The prophase or stage of preparation.** When the nucleus starts to divide the linin and chromatin combine into a single thread, which is wound or looped about the nucleus just within the nuclear membrane. This thread is sometimes called the **spireme,** or **skein.** Has the nucleus changed in size?

The thread of chromatin and linin breaks up into a number of segments called **chromosomes.** The number of chromosomes is constant for each species of plant or animal, but varies in different species. While this is going on, the nuclear membrane disappears, and a **spindle** figure appears in the position occupied by the nucleus, with fibres converging at two opposite points called the poles. This spindle commonly originates in the cytoplasm. The chromosomes arrange themselves at the equator or widest part of the spindle.

3. The **metaphase, a stage of division.** The chromosomes now split longitudinally into halves and the halves are pulled apart.

4. **Anaphase, a stage of separation.** The half chromosomes then separate, one half of each going to one pole, the other half of the same chromosome to the opposite pole. Note that by this division and separation, the number of half-chromosomes at each pole is the same as the number of whole chromosomes in the preceding nucleus, and that all kinds of material composing the chromosomes, nucleo-proteins, are likewise distributed to the new nuclei to be formed.

5. **Telophase, the end phase, and reconstruction.** At the poles the two groups of half chromosomes are reconstructed into nuclei, first forming a thread stage more or less like the early prophase; and finally the two new nuclei go into the resting stage, as at the beginning, and the whole process is complete so far as the nucleus is concerned.

B. **Cell division.** During the reconstruction of the nuclei at the end of mitosis, the preparation for the division of the cell into two cells takes place. Between the two nuclei and across the widest part of the spindle, a row of granules becomes visible, apparently beginning as thickenings of the spindle fibres. These granules finally fuse to form a continuous plate. The spindle widens, and the **cell plate** widens with it till it reaches the walls of the parent cell. The cell plate then splits and the new cell wall is formed between the two halves. When it has reached the old cell wall at all points of its circumference, the cell division is complete, the nuclear division having been followed by cell division. In this process the spindle fibres originate in the cytoplasm, and finally become a part of the cytoplasm again.

Notice that nuclear and cell division take place only in a short region near the tip, and as these divisions become fewer, cell elongation or growth in length occurs. The whole length of your sections probably includes the combined dividing and elongating zones. What is their actual length?

Differentiation has begun in this part of the root. It is completed in a zone farther from the tip. You saw the dermatogen, the periblem, and the plerome. In the latter, the very large cells have begun the differentiation by which, finally, they become the large vessels of the xylem such as you saw in cross sections of the mature root.

In the tip of every root, and in stems as well, nuclear and cell division, growth, and differentiation are going on. They are the three processes which determine the development of every individual.

Physiology of Roots.

1. Roots of a species of plant are marked with india ink in millimeter spaces beginning at the tip. These are then placed in moist air under conditions favorable to growth, and allowed to stand for some hours or a day or more.

Note carefully how many of the marked spaces have increased in width, and which ones have increased the most. You are determining the zone of greatest elongation, as well as the total length of the elongating zone.

Compare these observations with those made on the seedling roots with root hairs; and with the longitudinal sections of the root tips. Show the agreement among all these observations. What disadvantage would it be to have root hairs on the elongating zone?

2. **Demonstration of osmosis.** In an osmometer, molasses, which is a concentrated solution of sugar and other substances found in the sap of the sugar cane, is separated by a

semi-permeable membrane from tap water, which is a very dilute solution of various salts. The experiment is started with the two solutions at the same level. What change has taken place when you observe it? In which direction does the water move faster, toward the more dilute or more concentrated solution? In a similar way root and root hair absorb water because their cells contain a more concentrated solution than that of the soil water. Is there any evidence that dissolved substances, solutes, pass through the membrane in the opposite direction from that in which the greater flow of water takes place? Compare with experiment No. 3.

3. Notice a slice of red beet which has lain in water for a few hours or a day, after the red sap from the cut cells has been washed away. Compare it with another slice of the same beet, also in water, but first heated to the temperature of boiling water for a few minutes. Why does color diffuse out of one and not out of the other? This shows that while the roots of plants easily take up water, as long as their protoplasm is alive they can hold back the substances dissolved in the cell sap, as the red pigment is held back in the live beet. When the protoplasm dies it no longer acts as a semipermeable membrane, but is perfectly permeable. Hence the coloring matter and other substances diffuse outward.

4. **Plasmolysis.** Apply a drop of a 5% salt solution to a leaf of Elodea, and watch the effect upon the individual cells. Note particularly the changes in the position of the protoplasm, and the size of the vacuole. Explain this action on the basis of osmosis, considering the protoplasm as the semipermeable membrane. What goes out of the vacuole? Why? Show how this principle works when plants take in water by osmosis.

5. Observe some roots which were laid in a horizontal position after they were several centimeters long. What change has taken place? How long has the time been?

6. Compare the roots in the last experiment with others of the same kind, also laid in a horizontal position, but kept revolving slowly, also with a third set allowed to stand in a vertical position. What is the result? What conclusion can you draw from the last two experiments as to the form of energy which stimulates the roots to grow downward. Show the logical connection between the facts and the conclusion.

What advantage to the plant is this reaction? Note the different mode of reaction of roots and stems to the same stimulus.

Modification of Stem, Leaf, and Root.

While roots generally serve for absorption of water and minerals; stems for conduction of water, minerals and food, and leaves for photosynthesis and transpiration; yet all three of these organs may be modified for other purposes. In the pea a leaflet is modified into a tendril, while the tendril of the pumpkin is a branch of the stem.

In asparagus, the leaves are reduced to mere scales, while the stem carries on photosynthesis. In the ornamental Asparagus plumosus (improperly called the asparagus fern), the chlorophyll bearing stems are cylindrical. In Asparagus Sprengeri the stem is modified into a form like linear leaves, and in Myrsiphyllum asparagoides, (the smilax of the florist), the modification of the stem is like a small ovate leaf. What is the evidence that these are stems and not leaves? Draw a fragment of each of these species.

Bulbs and tubers are also modified stems or combinations of stem and leaf. Note the hyacinth bulb, or the tulip bulb, and the crocus corm, cut through vertically. Describe and draw so as to show what part is stem and what is leaf or leaves. Study the potato tuber. It is a modified stem. The eyes are the buds, each in the axil of a diminutive scale leaf. Draw to show the characters. Look at a potato cut across and find the vascular bundles. Which part of the stem is thickened most, the pith or the cortex? Draw.

KINDS OF FOOD.

The diet of animals is commonly said to consist of carbohydrates, fats, proteins, and minerals. Plants require these also for food, but green plants are able to synthesize the carbohydrates, fats and proteins; while non-chlorophyll-bearing plants, if furnished a suitable carbon-compound, can synthesize fats and proteins. Animals must have all three kinds of food furnished to them ready made.

A. Scrape the cut surface of a potato with a scalpel or other edged instrument and mount the scrapings in water. Study under low and high power. Study the larger granules. What is their shape, size, color? Under high power note their internal structure. A stratification may be seen, and a point about which the deposition of layers started. Is this centrally or excentrically placed in the grain as a whole? Draw several grains to show range of size, shape, and structure. Place iodine at the edge of the cover and let it diffuse under. What are the grains? How do you know?

B. You saw **starch grains** present in chlorophyll bodies in Elodea leaves, and you may now look at sections of **Pellionia stem,** in which much larger grains are stored, though not as large as the largest potato starch grains. In the outer layer of Pellionia you will see chloroplasts without starch, or with only very small starch grains in them, while in the deeper-lying cells starch grains appear larger as the cells lie deeper. Note that the chloroplasts are situated mostly at one side of the large starch grains, but a thin layer surrounds each grain. Draw several stages to show development of the starch grain. Just as the **chloroplasts** store up starch in Pellionia, so the **leucoplasts** store it in underground parts of plants, as the potato tuber; but the leucoplasts are smaller and it is much more difficult to see them than to see the chloroplasts. Leucoplasts are able to change sugar to starch, but cannot make sugar out of carbon dioxide and water.

C. Mount starch grains from the disintegrating endosperm of a germinated grain of corn. Notice the different states of solution of starch brought about by the enzyme, diastase. The starch is thereby changed to sugar, and in this form is transported from cell to cell. Starch grains, being solid, cannot be transported through cell walls or protoplasmic membranes. The change of starch to sugar by an enzyme is what happens in the digestion of starch in animal physiology. The ptyalin in the saliva of human beings is such a starch-dissolving enzyme.

D. In the seed of **Ricinus communis,** the castor bean, the **protein** is stored in the form of large **aleurone** grains. This seed also is the source of one of the oils, **castor oil.**

1. Mount a drop of alcohol which has been standing over crushed castor beans. It will contain castor oil in solution

and aleurone grains in suspension. Examine the latter quickly as the alcohol will rapidly evaporate and the oil will interfere with the view of the aleurone. These grains are more uniform in shape and size than the starch grains studied. They consist of a **ground substance** within which is found a spherical body called the **globoid,** and a crystal of protein, larger than the globoid. This is called the **crystalloid.** If the crystalloid cannot be made out, add a very small drop of iodine solution to the edge of the cover and let it diffuse under slowly. Watch the aleurone grains ahead of the iodine and as the iodine reaches them, the ground substance partly dissolves, and the crystalloid and globoid both appear more clearly. Crystalloid and ground substance are both proteins, and the globoid is an organic compound of calcium and magnesium. Note the color of the crystalloid due to iodine. Contrast this with the color of starch treated with iodine. If the iodine treatment does not show the crystalloid, add a drop of potassium ferrocyanide to a fresh mount of the alcohol standing over castor beans. This will dissolve the ground substance completely leaving the globoid and crystalloid lying side by side but free.

2. Another sample of alcohol that stood over castor beans has had a solution of Sudan III added. Mount a drop of this in a little water. The **oil,** which stays in solution in alcohol, is thrown out of solution by water, and appears as drops or smears of various sizes stained red by the Sudan III.

Describe them.

Other seeds yield oil, as corn, cotton, flax; also fruits, as the olive, anise, and caraway.

E. **Volatile oils.** Take a piece of fresh orange peel, hold it near a flame, bend it sharply between the thumb and finger. A fine spray of oil is emitted which burns in the flame. Notice its odor and taste. This is an example of a volatile oil, of which many are used in pharmacy and medicine, though not as foods. Plants whose leaves are odorous often secrete volatile oils in hairs on the surface. Peppermint is one example, the sweet-scented geranium, another. Crush a little of a castor bean on paper and notice the spot made. Spray the volatile oil of the orange on another piece of paper. Does either or both produce a permanent spot? An oil which leaves a permanent spot on paper is called a "fixed oil." One that evaporates is a volatile oil. Is castor oil a volatile or fixed oil?

CHEMICAL ELEMENTS NECESSARY FOR LIFE.

When plants are analyzed chemically, they are found to contain between 15 and 20 of the chemical elements. But the question as to whether these are all absolutely necessary can be determined only by experiments, and these have shown that they are not all necessary.

A. For Fungi and other plants without chlorophyll, a carbon compound must be furnished, while green plants make their own carbon compounds, (sugar). In either case part of the sugar or other carbon compound is oxidized to furnish energy for synthesizing fats, proteins, and other substances found in plants, and part is needed to furnish carbon, hydrogen, and oxygen for the new chemical combinations.

Culture media are made up with and without certain chemical elements; and fungus spores, the reproductive bodies of fungi, are inoculated into these media. A complete culture solution is made up as follows:

1. Monopotassium phosphate, KH_2PO_4	.2 gm.
2. Magnesium sulphate, $MgSO_4$	.02 gm.
3. Calcium nitrate, $Ca(NO_3)_2$	.2 gm.
4. Cane suger, $C_{12}H_{22}O_{11}$	5.0 gm.
5. Distilled water, H_2O	100. cc.

How many and what different elements are contained in this medium?

Modifications are made as follows:

a. Sugar is left out entirely; What element is then lacking?

b. Calcium is left out by substituting potassium nitrate, KNO_3, for calcium nitrate;

c. Phosphorus is left out, substituting potassium nitrate for potassium phosphate;

d. Nitrogen is left out, substituting calcium sulphate for calcium nitrate.

In substituting, no element was added which was not in the complete solution, and not more than one of the elements contained in that solution is left out of any other one.

Conclusions can be drawn as to the necessity for each element left out, allowing for the fact that the fungus makes a small visible growth even in distilled water, using the food stored in the spores.

Compare the relative amounts of growth in the different solutions. Draw conclusions as to the necessity of the elements left out.

B. Testing whether the same elements are necessary for green plants as for fungi. Since green plants make their own sugar, no sugar is put in the culture medium for them. To the complete solution used for fungi except sugar, a few drops of an iron salt, ferrous sulphate, Fe_2SO_4 were added. With this addition and without sugar there were used:

1. The complete solution;
2. Calcium left out;
3. Nitrogen left out;
4. Iron left out.

Note what species of plants were used, the length of time necessary to show differences, and the effect on growth of the lack of chemical elements left out. What elements tested do green plants require that fungi do not? It is to be noted that the food stored in the seed is sufficient to support the growth of the plant for a limited time.

Experiments similar to the above, carried out with the most extreme care as to purity of chemicals, etc., have shown that for some fungi only the following elements are necessary: **carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium,** and **magnesium.** The first three are found in **carbohydrates,** the first five in all **proteins,** and the first six in **nucleo-proteins.** The last two are metallic elements. In addition, **calcium** and **iron** are necessary for most, if not all, green plants; and **sodium, chlorine,** and **iodine** are **required** for the **higher animals.** Perhaps **fluorine** and **silicon** are also requisite. The former is a constituent of bones and teeth, and the ash of hair, horns, hoofs, and feathers, is nearly one third silica.

RESPIRATION AND THE RELEASE OF ENERGY

In growth and other physiological processes, plants require energy as animals do. The primary source of their energy is, of course, the sun's energy used in photosynthesis. In this process the active, or **kinetic, radiant energy** of the sun is changed to the **potential,** or stored energy, in the form of the **chemical energy** which binds together the atoms in the sugar molecules. In order that this stored energy may be used for the physiological processes necessary for life, it must again be released, or made active, for further transformations. This release of energy is brought about by the process of respiration, a process which is essentially alike in both plants and animals Carbon compounds, sugars and others, are decomposed into simpler ones, CO_2 and H_2O being two of the end products of an oxidation process. Oxygen is required to carry on this oxidation. The process takes place in all living cells and releases the energy stored in the substances decomposed. The released energy appears partly as **heat,** a form of kinetic energy. It may be utilized as heat, maintaining a definite temperature, as in warm-blooded animals; or it may be transformed by the organism into light, electricity, mechanical motion; or as chemical energy it may be stored in other newly-formed substances. All organic compounds have a certain amount of energy stored in them, and directly or indirectly this is practically all transformed radiant energy.

The energy value of foods is expressed in heat units called **Calories.** A small and a large Calorie are used as units. The former is the amount of heat required to raise the temperature of 1 gram of water from O°C. to 1°C. under standard pressure. The large Calorie is equal to 1000 small ones, and is the amount of heat required to raise the temperature of 1000 grams of water (1 kilogram) from O°C. to 1°C. The large Calorie is used to express the energy value of diets. The energy in a given quantity of food may be determined by burning, that is, by combustion, and measuring the heat evolved. The value as determined by combustion is not necessarily the amount of energy available to the living body, for two reasons: 1, foods are not always entirely digested by animals; and 2, when digested and assimilated, they are not entirely oxidized in respiration. For example, proteins, in respiration in the animal body, leave the residues urea and uric acid, both of which are capable of further oxidation, thereby releasing some energy.

1. Germinating seeds may be used to show that respiration releases energy as heat. They must first be soaked in water, since a large amount of water is necessary for growth. They should be placed in a chamber insulated so as to prevent so far as possible the loss of heat by radiation. Such a vessel is a Dewar flask, with double walls between which is a high degree of vacuum. (It is on this principle that thermos bottles are made.) An ordinary thermometer inserted among the seeds will serve to show the difference in temperature between the flask containing live germinating seeds and another similar flask containing seeds killed by boiling and therefore incapable of growth. This second flask is the check, or control. What is the difference in temperature? The heating of cereal grains stored in large quantities in too moist a state is another exhibition of the heat released in respiration, as is also the so-called "sweating" of hay or grain in stacks. Under such circumstances, the amount of heat generated may be actually sufficient to scorch the grain or even set fire to the hay by "spontaneous combustion," but respiration alone cannot account for all the heat.

2. It is well known that if a human being exhales through a glass tube into a bottle of lime water, (calcium hydroxide) the CO_2 in the respired air will combine with the lime to form the carbonate of lime ($CaCO_3$) which, being insoluble in water, shows as a white precipitate.

To test whether respiration of plants is like that of animals, place seeds, leaves, or opening flowers in a closed bottle or flask, and after they have stood for some hours in the dark, draw the air in the bottle into lime water, or baryta water (barium hydroxide). Is a precipitate formed? Is respiration in plants like or different from that of animals as regards the production of CO_2? What check, or control, is necessary, if the conclusion from this experiment is to be valid?

3. Lower a lighted splinter into a bottle set up as for experiment No. 2 and kept in darkness. Is it extinguished or does it burn more brightly? What conclusion can you draw from the observed result? Be careful not to draw any conclusion not warranted by the facts.

ENERGY IN DIET.

On the average, 1 gram of carbohydrate has a combustion value of 4.15 Calories, and yields 4.03 Calories to the human body (Atwater's results). One gram of protein has corresponding values of 5.65 and 4.05 Calories, and 1 gram of fat yields 9.40 and 8.93 Calories. It is seen from the above figures that, weight for weight, fat has more than twice the energy value of carbohydrate and protein in the living body, while the latter two are practically equal. This explains why fat enters so largely into the diet of inhabitants of cold regions. The number of Calories required per day for the average individual, as determined by Atwater, is 2450 for a woman at light work or a man without muscular exercise; and up to 5500 Calories for a man at very hard muscular work.

The standard set by Hutchison for a man of average weight at moderate muscular work, is 3000 Calories per day. When people are allowed to choose their own diets freely, it has been found that they take about 120 grams of protein, and Hutchison (Food and Dietetics, 1916) accepts that as the proper standard for the protein which is necessary to nourish the living protoplasm. The balance of the diet should be made up of carbohydrates and fats, and these will vary according to the taste of the individual. In the standard named, 500 grams of carbohydrates and 50 grams of fats are given as the proper proportion. The amounts just given furnish a little less than 3000 Calories per day. Chittenden expressly advocates half this quantity of protein, namely 60 grams per day, and a total of 2800 Calories, for the average man at moderate work. This "low protein diet" is advocated on the basis of experiments carried out on professional men, athletes, and soldiers, who maintained a nitrogen balance and body weight with ability to work for some considerable time on the 60 grams of protein per day.

If it were certain that the low protein would be adequate for all persons, at all ages, and throughout life, it would be a distinct advantage; since protein is more expensive, both financially and physiologically, than are fats and carbohydrates. The adequacy of the low protein diet may be doubted, however, partly for reasons that Hutchison has discussed; and in the next paragraph it will be pointed out that mere quantity of protein is not sufficient. It must be adequate in quality as well. It seems wiser to have a "margin of safety" rather than a balance determined by experiments, which, at the most, can be continued for a period of time relatively short.

QUALITY OF PROTEINS AS FOOD.

For nutrition of the protoplasm mere **quantity of protein** is not sufficient. It must be **adequate in quality** as well, and quality is determined **by the amino acids** which a protein yields on complete digestion. Eighteen to twenty amino acids are known as products of protein digestion. Recent experiments on feeding animals have shown that to nourish the protoplasm of mammals, a certain group of five or six amino acids is indispensable. All of these must be present. Very few, if any proteins of seeds yield all of these amino acids. One or more is lacking in practically all the seeds tested, perhaps in other plant proteins as well. Just which ones are lacking has been determined for so few proteins, that it is hardly possible as yet to give general rules as to which natural foods supplement each other. However, milk proteins supplement those of some seeds, and our use of milk with cereals undoubtedly has a sound basis in the chemistry of protein. Until our knowledge is more complete **the wise course is to supply our proteins from a variety of sources,** and in an excess over the minimum as determined by short-time experiments, on the **probability that we shall** in that way **get the necessary assemblage of amino acids.** The vegetarian in particular is in danger of a lack of one or another amino acid. Animal foods, such as beef, pork, mutton, etc., having already been synthesized by mammals, may be supposed capable of yielding on digestion the amino acids suitable for re-synthesis into other mammalian protoplasm.

MINERALS IN FOOD.

Our new knowledge of nutrition has shown the importance of certain **mineral constituents** in foods. Animals, fed on diets purified to a degree suitable for experiments on nutrition, must be supplied with a suitable salt mixture to maintain health. One school of investigators uses in the experimental diets a "shot gun dose" of **acids, alkalies,** and **salts,** containing all the chemical elements that organisms are at all likely to require. Another leading worker in this field says that **sodium, chlorine,** and **calcium,** are the mineral elements most necessary in quantities above those found in most foods, and adds to his experimental diets 1 per cent sodium chloride ($NaCl$) and 1.5 per cent calcium carbonate, ($CaCO_3$). A salt of iodine is added at intervals.

It is well known to the medical profession that a form of goiter, commonly called "big neck," because of the enlargement of the thyroid gland in the neck, is prevalent in certain regions of the earth. One such region is the vicinity of the great lakes in North America. It has been suggested that the prevalence of this form of goiter is due to a **lack of iodine** in the food and water of these regions, since iodine is necessary for the normal functioning of the thyroid gland, and is used in the treatment of this form of goiter.

In a certain city of Ohio, a study was made as to the prevalence of goiter among several hundred school children. They were then treated with sodium iodide twice a year, in spring and autumn, for a period of ten days, and the condition noted in the following years. This condition was much improved, both as to the number of children having goiter and as to the degree of enlargement in those in whom it had not disappeared.

One writer has recently suggested that the refinement of our table salt at the present time is a detriment, in that formerly it contained sufficient **calcium** to supply the lack of that element in an ordinary diet; but that the purified salt now commonly used on the table no longer supplies the requisite amount of calcium.

If this is correct, the remedy would seem to be either the use of a cruder grade of salt for **cooking** purposes, or the addition of a **calcium** salt as well as **sodium chloride** to the diet. It is a fact that some cruder grades of salt taste "sweeter," than the refined grade.

Iron is a constituent of the blood proteins, the haemoglobins. It is sometimes believed to be deficient in human beings and iron-containing tonics prescribed.

Minerals are not always regarded as foods, this term being often restricted to energy-furnishing materials, such as carbohydrates, fats and proteins. But minerals furnish some chemical elements necessary for structural purposes, as well as for maintaining equilibria of physical and chemical processes. They are foods in the general, but not in the restricted sense.

VITAMINS.

In the 20th century it has been found that fats, carbohydrates, proteins, minerals, and Calories are not the only factors requisite for growth of young animals or the good health of adults. Feeding experiments with animals together with a study of certain diseases of human beings have shown the necessity for another class of substances, whose chemical composition is entirely unknown, but which are known by the disease or degenerative condition that occurs in their absence.

These substances have been called **Vitamins,** and three different ones have been distinguished with a reasonable degree of certainty:

1. **Fat-soluble A,** the anti-xerophthalmic vitamin or anti-rachitic vitamin.

2. **Water-soluble B,** the anti-beriberi or anti-neuritic vitamin.

3. **Water-soluble C,** the anti-scurvy or anti-scorbutic vitamin. In the absence of a certain quantity of all or any one of these, young animals soon cease to grow, lose weight, and finally die, if the deprivation is too long continued. Also the absence of each one leads to the development of a certain diseased state, more or less peculiar to each vitamin. It is by means of these that they have been distinguished from one another.

The first is found associated with fats and oils, in which it is believed to be soluble, but is not soluble in water. In its absence a certain degeneration of the eye occurs which has been named xerophthalmia. It involves an inflammation and hardening of the eye-lids, and a degeneration of the cornea and optic nerve, leading to complete blindness. It is the probable cause of frequent blindness in orphanages in which, as has happened in Denmark, children are fed too largely on skimmed milk, with too little of the cream or butter fat, which is rich in this vitamin. It is also abundant in egg yolk, and is fairly abundant in the fat of internal organs of animals, as the heart, liver and kidney; but the fat of muscle, or ordinary meat, has little of it. Cod-liver-oil is an excellent source of this vitamin and is valuable in the treatment of the malnutrition that occurs in its absence.

Apparently, animals are not able to synthesize any of the vitamins. They can only concentrate what they get in their plant food. The cow concentrates fat-soluble A in the butter fat; the hen in the egg yolk; and it is also found in some oily seeds, but the extracted oils, as well as the rendered animal fats, such as lard and tallow, have almost none.

It is believed that the disease known as rickets, or rachitis, is the result of malnutrition associated with the absence of the fat-soluble vitamin, which has therefore been called the anti-rachitic vitamin. The latest experiments indicate that a condition hardly distinguishable from rickets except in the microscopic structure of the bones, may be caused in rats by a deficiency in phosphorus, in calcium, or in the fat-soluble A. The most available sources of fat-soluble A for human beings are butter, eggs, leaf vegetables, and the fats of internal organs of animals.

The second vitamin, **water-soluble B,** is distinguished by the fact that in its absence a disease known as **beri-beri** is produced in man, and a similar disease called **polyneuritis,** in animals experimented upon. Lameness, emaciation, and degeneration of numerous nerves occur. The disease is found always associated with very restricted diets, and especially in the parts of the world where polished or hulled rice forms too large a proportion of the total diet. The condition may be brought about in animals fed too exclusively on any cereal from which the bran and the germ are removed, as they are in highly-milled, white wheat flour. The cereal grains, rye, wheat, rice, etc., contain a moderate amount of the water-soluble B vitamin, but it is nearly all in the outer layer of cells, just within the seed coat, and in the germ or young plant in the seed. Both of these are removed in milling the wheat and hulling the rice also deprives it of most of its vitamin content. If diets must be restricted largely to any of these cereals the whole grain should be used instead of the milled or hulled grain. The anti-beri-beri, or anti-neuritic vitamin, is rather widely distributed in both animal and vegetable foods; and the safest diet is one of considerable variety, including the

leaf vegetables, as well as certain root vegetables. **Cabbage, onions, carrots,** and **rutabagas** are rich in it, while beets are very poor. It is abundant in yeast, but since it is also widely distributed in many other foods, the indiscriminate use of yeast as a food is of more than doubtful value. Certain undesirable results are likely to follow its extensive use, not to say dangerous results. It should be used only on the advice of a physician.

The third vitamin, **water-soluble C,** prevents scurvy, which develops in its absence. This disease has long been known among sailors, and the sailors and soldiers in the great war did not escape it. It occurs when dried or canned foods, of whatever kind, form a diet too exclusively. In the days of the old sailing vessels, it was learned that the disease could be prevented if fresh fruits and vegetables were available for food. Especially useful was the juice of one of the citrus fruits, called the lime. In the recent war, however, lime juice was found almost useless. This was explained when an historical research showed that the fruit called the lime in the olden days was the one known to us as the lemon. Both **oranges** and **lemons** are very rich in the anti-scurvy or antiscorbutic vitamin, and other fresh fruits and vegetables have considerable amounts. Here again, **cabbage, onions,** and **carrots** are valuable sources, but **they must be eaten raw** to obtain this vitamin in suitable quantities. The anti-scorbutic vitamin is easily destroyed both by drying and by heat. Hence dried, canned, or cooked foods are practically valueless as sources of it. Some foods containing it should be eaten raw. Even the temperature at which milk is often pasteurized, namely 60-65°C. for one half hour, is sufficient to destroy it. It is less completely destroyed if the milk is heated to a higher temperature, even to boiling, for a short time, and, especially if it is heated in a closed vessel. The destruction seems to be dependent upon a process of oxidation, which is prevented, or at least reduced, if the milk is not in contact with the air while heating. For children who must be fed on heated milk, orange juice is an easily available source of the anti-scurvy vitamin.

Under present conditions, it is practically necessary to heat or pasteurize milk to make it safe, especially for babies, against infectious diseases, particularly tuberculosis; but occasionally also against typhoid fever, diptheria, scarlet fever, or other diseases.

To sum up, we may say vitamins are substances necessary for growth and health, in addition to fats, carbohydrates, proteins, and minerals, and the required energy. They are of at least three kinds, each distinguished by a particular disease, a degenerative state, which occurs in its absence. All three are more or less abundant in leaf vegetables and in some root vegetables, cabbage, onions, and carrots being especially useful sources, easily available. Butter or unskimmed milk, eggs, the fat of internal organs and, in emergencies, cod-liver oil are sources from the animal kingdom for the fat-soluble A. Water-soluble B, the anti-beri-beri or anti-neuritic vitamin, is widely distributed in both the plant and animal kingdoms and the best protection against an insufficiency is a wide variety of food, especially fresh vegetables, which may be eaten either cooked or raw.

Water-soluble C, or the anti-scurvy vitamin is found in many fresh fruits and vegetables, and to obtain this vitamin they must be eaten raw. It is especially abundant in oranges, lemons, tomatoes, cabbage, onions, and carrots. The other two vitamins are much more resistent to heating, and are not destroyed in cooked, canned, or dried foods, provided they were present before cooking or drying.

In a recent book called "Studies in Deficiency Disease," 1921, McCarrison has shown that many forms of vague ill health or diseases whose cause has always been obscure may be due to partial deficiency of one or more vitamins, even when the deficiency is not complete enough to produce the specific diseases named above. He believes the partial deficiency of vitamins is more detrimental if at the same time the diet is proportionally too rich in carbohydrates. Among the forms of ill health he names as possibly due to this cause are, loss of appetite, forms of indigestion, colitis, ulcers of the digestive tract, defective teeth, imperfect calcification of teeth or bones, rickets, soft and bleeding gums, hemorrhage from any of the mucus membranes or other organs, cardio-vascular depression, anemia, and numerous others. Not only are there direct effects of the partial deficiency but there are also indirect effects through the action on the ductless glands, the adrenal bodies, thyroid, thymus, pituitary body, etc., glands whose products are known to control to a large extent health, growth, and development, both physical and psychical. McCarrison further believes that the condition of partial deficiency prevails over a much greater portion of the earth than any previous writer has supposed. It is prevalent not merely in those countries where the population is in a chronic state of semi-starvation but even in Europe and America, and among well-to-do people, much of the vague ill health is due to this cause. This view gets partial confirmation in the findings reported in various studies of the physical condition of American school children, among whom it is reported that malnutrition is by no means confined to the children of poverty-stricken parents. It is also common among the children of the rich.

These facts, if facts they are, are most startling. Moreover they are most hopeful. If these widely spread forms of ill health whose cause has been previously unknown are due, either directly or indirectly, to faulty diet, they can be controlled by a properly selected and balanced diet. Preventive medicine has a new weapon at its command. Also McCarrison has pointed out that sufferers from a faulty diet are less resistant to the pathogenic bacteria than people properly nourished. It is well known that one of the most important factors in the cure of tuberculosis is a rich, bountiful diet. Undoubtedly those perfectly nourished will have a high degree of resistance to many pathogenic organisms. Is not this the knowledge which when diffused among the population generally, will again extend the span of human life and make it happier by removing an appreciable part of the burden of disease? Can not the high percentage of physical unfitness revealed by the war be much reduced?

In the early part of the nineteenth century, at a time when the question of the food supply of Europe was almost as acute as now, owing to the Napoleonic wars, it came to the notice of the German medical profession that a family in Bavaria was very remarkable for the longevity of its members. Several lived far beyond the age of one hundred and one attained the astonishing age of one hundred and thirty five years. An inquiry was made as to the mode of life of this family. The only peculiar thing about it was that they grew, and largely lived upon, six different varieties of cabbage. The isolated instance of one family is of limited value as proof of a general proposition, yet taken in the light of our new knowledge it is perhaps significant. Some years ago Metchnikoff pointed out the fact that the Bulgarians were remarkable for the large proportion of aged persons among the population, and ascribed this fact to the extensive use of fermented milk in the diet of the people. Metchnikoff's interpretation of how the sour milk diet brought about longer life has not wholly stood the test of later criticism, but with the acquisition of the knowledge of vitamins his observation on the diet of the Bulgarians takes on a new significance. It is now clear for the first time that the common factor in a diet composed largely of sour milk and one of cabbage is the presence of an abundant supply of vitamins in both.

It is interesting also that the apparent contradictions between the dietaries of different peoples can now be harmonized. The Bulgarians get their mineral, protein, and vitamin balance in their favorite sour milk. The Chinese, who never use milk, get the same dietary elements in leaf vegetables.

It appears not improbable that the craving for alcoholic drink is not wholly the need of a drug to which the addicts have become accustomed, but is in part, a need of the body for more perfect nutrition, and this craving, so far it is not a willful desire to have what is forbidden, might be satsfied by a diet perfectly balanced for all the nutritive factors now recognized.

Summary for nutrition.

It seems extremely probable that much of the ill health which is without definite symptoms of specific disease, both in children and in adults, may be due to the lack of one or more factors necessary to perfect nutrition not included in those long known ones, the minerals, fats, carbohydrates, proteins, and Calories, or not sufficiently appreciated until recently. These deficiencies may be:

1. A lack of calcium, of iodine or of iron in the mineral constituents of the diet; or possibly of some other element, or a group of elements in proper balance;

2. A lack of proper quality of protein, as regards one or more of the requisite amino acids;

3. A lack of one or more of the three vitamins, fat-soluble A, water-soluble B, and water-soluble C.

THALLOPHYTA.

The whole kingdom of plants is divided commonly into four divisions:

1. **Thallophyta,** the thallus plants;
2. **Bryophyta,** mosses and liverworts;
3. **Pteridophyta,** ferns and fern allies;
4. **Spermatophyta,** the seed plants.

Our study so far has been on the structure and activities of the last-named division. They are the most complex of all the plants, and among them are the plants most useful directly to man as sources of food and of materials for clothing, for shelter, for furniture, and for other uses.

We shall now go to the other extreme of the plant kingdom, and take up the study of the simplest plants.

The division **Thallophyta** includes plants ranging in size from single cells barely visible with the most powerful microscopes, some of the bacteria, to plants several hundred feet long, some of the marine algae. They are of many degrees of complexity in **external form,** single cells, loose masses of cells, sheets of cells, simple filaments, branched filaments; and still more complex, a few resemble superficially the stems, roots and leaves of higher plants. They do not, however, have true stems, roots and leaves, and a **thallus** is any plant body which is not differentiated into those organs. In **internal structure** most of them have no trace of any vascular bundles, and no need for such structures, though a few of the large forms have something like sieve tubes. In **methods of reproduction** they vary from those which multiply only by cell division to methods of spore formation, both asexual and sexual, and finally, some, such as mushrooms, toadstoods, and puff-balls, form complex fruiting bodies.

In all of these respects, external form, internal structure, and methods of reproduction, the Thallophyta are simpler on the whole than any other division of the plant kingdom.

This group is commonly divided into **two subdivisions**:

Algae, containing chlorophyll, and

Fungi, without chlorophyll.

Each of these subdivisions is in turn divided into several classes. It is expected that the student will learn the names and characteristics of all the classes.

One of the classes of the fungi is the

SCHIZOMYCETES. Bacteria.

The bacteria are essentially **unicellular**, and are the smallest of plants in size, ranging in **diameter** from 0.1 micromillimeter to rarely more than 2 or 3 micromillimeters or microns. (1 micron is 0.001 millimeter, or 0.0001 centimeter. This is the standard unit for microscopic measurements.) In **length** the cells are mostly from 0.2 to 4 or 5 microns, though in a few species they are much longer, even 100 microns or more. In **shape** they vary from spherical to rod-shaped and spiral. In **structure** they are composed of a cell wall that is not pure cellulose, but contains nitrogen and resembles the animal substance, chitin; cytoplasm in the interior may be separated from this wall by a concentrated salt solution; and in this respect the bacterial cell is like the plant cell rather than the animal cell. There is no definite nucleus, and in this, bacteria show a simpler structure than most plants, but nucleo-protein, or chromatin, is scattered in the cell cytoplasm.

Some bacteria have the **power of locomotion** by means of one or more **flagella**, while others have no such power. **Reproduction** is by cell division, or fission, a cell dividing across the middle into two of equal size. Some bacteria form spores, but these are not generally a means of multiplication, since only one spore is formed by each individual cell. The spores are an adaptation to conditions unfavorable to life. They can withstand conditions that would kill the vegetative cells, such as drying, high temperature, treatment with germicides, etc.

Perhaps the two most significant characteristics of the bacteria are their **small size** and the **rapidity** with which they **reproduce.** The small size allows an enormous number to be present in a small space. Sour milk may contain in one cubic centimeter one billion of bacteria 1. micron long, and yet have only less than one-thousandth of the total space actually occupied by the bacteria. Another result of the small size is that they have a very great amount of **surface in proportion to their volume**; and the rate at which they bring about chemical reactions is proportional to the total surface rather than to the volume. A single cube, one centimeter on each edge, has a total surface of 6 square centimeters. If the same volume is divided up into small cubes each edge of which is only 1 micron in length, while the total volume is the same, the total surface is 10,000 times as great, or 60,000 square centimeters.

In rapidity of reproduction a few species are reported to show the surprising rate of one division in fifteen minutes; others require a half hour; and one division per hour is well within the reproductive capacity of many species, when under conditions favorable for growth. At this latter rate, continued uninterruptedly, a single bacterium will have nearly 17,000,000 descendants at the end of twenty four hours . If the rate of division were one every half hour, the total number at the end of twenty four hours would be the square of seventeen millions, and if division occured every 15 minutes the number after 24 hours would be the fourth power of seventeen millions.

These two characteristics, then, give to bacteria an enormous capacity to produce large effects in a short period of time, whether those effects are in the causation of disease, in the decomposition of organic matter, or in bringing about other chemical reactions.

In nature bacteria are usually found abundant in putrefying material, many kinds growing together in a mixed culture.

A. If such a culture is furnished, mount material from it and study the forms occurring therein. Note their minute size. What different shapes do you see? Are any of them in motion? Is it rapid or slow? Is it in straight lines or spirals?

Bacteria may be obtained in pure cultures, that is a culture in which only one species is growing. Such a culture is provided of

1. **Bacillus subtilis.** Make a note of the culture medium on which the bacteria are growing. The instructor will give you the constituents of the medium. Describe the appearance of the growth, color, consistency, character of surface. Following the directions of the instructor, make a mount for microscopic study. Examine under high power. What is the shape of the individual cells? If cells are attached to each other, how are they arranged? Are any cells in motion? If so, is it slow or rapid? In a straight or a spiral path? Is it both backward and forward? In a culture several days old, you may find spores. What is their size, shape, and density as compared with the active cells? Their position in the cell which forms them?

2. **Sarcina aurantiaca.** How does this form differ from **Bacillus subtilis,** as seen with the unaided eye? As seen under the high power, how does **Sarcina aurantiaca** differ from **Bacillus subtilis** in shape, size and arrangement of cells, locomotion and spore formation?

3. **Root tubercles** are inhabited by a species of nitrogen-fixing bacteria, Rhizobium leguminosarum, also called Bacillus radicicola and Pseudomonas radicicola. On the plant furnished you, clover, alfalfa, or pea, examine these swellings, tubercles, or nodules. How do they differ from the rootlets? Are they numerous or few? What is their size and shape? These nodules are caused by the presence of the bacteria cells within the cells of the clover, etc.

To understand the full relation of these nitrogen-fixing bacteria, read the account of the nitrogen cycle, pages 52, 53.

4. **Bacteria and Milk.** Milk, as obtained from the dealer, is set up in two series of experiments, one without heating, the other heated to various temperatures at the beginning of the experiment.

a. Flasks marked T are set at different temperatures, without preliminary heating, to find the effect of temperature on the time milk will keep without souring or spoiling. These temperatures will probably be 5°, 15°, 20°, and 37½°C. Note the time that elapses before souring at each temperature.

b. The second set is heated to various temperatures at first, then kept at room temperature, about 20°C. The flasks given the different treatments are marked with the symbols at the beginning of the following paragraphs.

P. This milk is first heated to 65°C. for ½ hour, then kept at 20°C. This treatment is one method of pasteurization. Pasteurization is intended to kill the disease-producing bacteria. Does it also kill the souring bacteria?

Bo. Heated to 98° (boiling point) ½ hour, then kept at room temperature.

S. Heated to 120°C fifteen minutes, then kept at 20°C. with stoppers. This is sterilized. All bacteria are killed.

SO. Heated to 120°C. fifteen minutes then kept at 20°C. without stoppers, that is, exposed to contamination from the air.

Sb. Heated to 120°C. fifteen minutes, then inoculated with Bacillus subtilis, and kept at 20°C.

Note that all flasks except So, are stopped with cotton stoppers which should not be removed. The flasks are not closed air-tight, but the stoppers filter all bacteria from the air that passes through them so long as they are dry, and thus prevent bacteria or mold spores from getting into the flasks.

The observations should indicate what visible changes occur and the earliest time at which they occur. The significant changes visible to the naked eye are chiefly three. The first is a curdling or thickening which indicates a sour state of the milk. Lactic acid is formed by bacteria at the expense of milk sugar, and this acid causes the casein of the milk, its most abundant protein, to change from a colloidal solution, to a gelatinous curd or in terms of physical chemistry, from a "sol" to a "gel." The sour milk may show a firm curd throughout, or a translucent liquid, the whey, may separate from the firmer curd. A second change that may be visible in the milk is a digestion of the casein. This may take place either with or without souring. The digesting or putrefactive bacteria have their growth inhibited by acid sufficient to cause curdling. Digestion or putrefaction is indicated by a gradual disappearance of the gelatinized curd of sour milk or of the casein of un-soured milk. In the latter case a brownish liquid appears as the casein disappears. A third visible change is a molding. Molds are visible as filamentous growths or as dense patches of color, black, brown, white, yellow, red, or green. Molds are fungi of a higher type than the bacteria.

After the observations are completed they should be written up, and the write-up should be given in four distinct parts: first, the experiments should be described; second, a summary of the observations in concise sentences; third, a discussion of the significance of the facts observed comparing the results in different sets; fourth, a series of conclusions based on these facts.

In the discussion compare one part of the experiment with another, and consider the following questions:

Which bacteria are more numerous and significant, those that are present in the milk on delivery, or those that gain access to it after delivery? In series T, which temperatures are best adapted for home use, and why? Compare results of heating to 65°, 98°, and 120°C. and indicate the advantage and disadvantage of each of these temperatures. Why not heat all milk to a temperature that would completely prevent the souring or spoiling of milk?

In drawing conclusions take into consideration facts given on the destruction of vitamins by heat.

Draw conclusions as to the following points:

1. What causes milk to spoil or sour?

2. Which are more important, the organisms present in the milk when it is delivered or those getting into it after delivery?

3. Should it be completely sterilized?
If not, why not?

4. Should it be pasteurized? If so, why?

5. Should it be taken without any heating? Give a reason for your answer.

6. After milk is delivered, what precautions should be taken in keeping it? Why?

SACCHAROMYCETES. Yeasts.

The yeasts are usually placed as an order under the scomycetes, but there are some advantages in studying them connection with the bacteria. They differ from the bacteria size and shape of cells, and in the method of reproduction.

Saccharomyces cerevisiae. Brewer's yeast. Bakers yeast.

A. Mount a portion of dried yeast cake in water. Note e different bodies visible. Try to distinguish yeast cells om other constituents. Now apply a drop of iodine to the lge of the cover glass. What substance makes up the bulk the yeast cake? The yeast cells can now be distinguished ore certainly by the color the iodine gives them. How do the erage cells differ in size and shape from bacteria cells? How uch difference is there between the largest and the smallest?

Draw a few cells to show variations in the shape and size.

B. Observe a sugar solution or fruit juice to which yeast s been added some time earlier. What do you see taking ace? What process may you infer? What gas is being pro-ced? Where does the gas come from? What is another oduct besides the gas? What common household process dependent on this same activity of yeast? What different e is made of the products in this latter process?

C. Mount a portion of the solution containing the active ast and note the difference between this and that in the y cake. How does the yeast multiply?

Show how this differs from the reproduction of bacteria. hat cell contents can you recognize?

Draw a chain of cells showing the mode of reproduction.

The Carbon and Nitrogen Cycles.

While some bacteria cause disease and in other ways are trimental to man, many others are not only beneficial to him t the work that they do is absolutely necessary to the con-uance upon the earth of life in general. This work concerns e cycles of the chemical elements necessary for life, that is, e series of chemical combinations into which these elements enter in serving the necessities of living organisms. These cycles are best presented in relation to carbon and nitrogen.

The carbon cycle. The carbon supply in nature available to plants is the carbon dioxide of the air. It is present in the air only to the extent of 0.03-0.04 of one per cent. Plants draw upon this supply in photosynthesis, making sugar of CO_2 and H_2O. C. G. Hopkins has calculated that a crop of corn yielding 100 bushels to the acre would exhaust the CO_2 in the air over an acre in 2 years if no new supplies of CO_2 were returned to that air· Carbon dioxide is constantly being returned to the air in the respiration of both plants and animals. Combustion of coal, wood, and other organic compounds returns another portion. Volcanoes and hot springs contribute a share. But in spite of all these sources of renewal of the supply of CO_2, the amount of carbon available for living beings would constantly grow less, if the waste products of plants and animals and their dead bodies did not undergo decay. Now it is primarily the work of bacteria and other fungi to bring about this decay. Bacteria are particularly active in the decomposition of proteins and other nitrogenous substances. Yeasts are especially active in the decomposition of soluble carbohydrates, as in the fermentations of sugar solutions, though many bacteria can also ferment sugars. The more highly organized fungi, as the Ascomycetes, the Basidiomycetes, etc., can not only decompose proteins and carbohydrates, but some of them can decay the very indestructible parts of organism, as the bones, horns, hoofs, hair, and feathers of animals, and other species are able to attack the cellulose, wood, cork, and cuticle of plants, substances which bacteria and yeasts are almost wholly unable to decompose.

This process of decay prevents the cumbering of the earth by the dead bodies and the waste products of plants and animals, and also sets free the chemical elements composing them for the use of succeeding generations of living beings. Thus, in spite of a limited supply, at a given moment, of carbon and nitrogen available to organisms, life upon the earth may continue for an unlimited time, so far as these and other necessary chemical elements are concerned, through the combined work of bacteria, yeasts, and other fungi on one hand, and the synthesizing activity of green plants on the other.

In a **short carbon cycle** the CO_2 of the air is utilized by the chlorophyll-bearing plant to make sugar, and some of the sugar may be oxidized at once to CO_2 and H_2O in the process of respiration, the energy released in respiration being used to do physiological work, and the CO_2 escaping to the air. The cycle may be longer than this, however. Instead of the sugar being immediately oxidized in respiration in the plant, it may be extracted from the plant and fermented by yeasts as in the alcoholic fermentation, only a part of the carbon being oxidized to CO_2, another part going into the alcohol produced. Then certain bacteria oxidize the alcohol to acetic acid as in vinegar making, and the same or other bacteria and molds oxidize acetic acid to CO_2 and H_2O.

In milk a somewhat similar process goes on. All unheated milk contains both **putrefactive** and **souring** bacteria. The putrefyers can slowly decompose or digest the casein, which is a protein, forming **peptones,** and the sourers decompose the milk sugar, forming **lactic acid.** When the acid has reached a certain concentration, the **casein is curdled**; but before this concentration has been reached, the action of the putrefyers has been stopped by the acid. After the milk has curdled, a mold, **Oidium lactis,** usually appears on it, and this mold can oxidize the lactic acid to CO_2 and H_2O. When the concentration of the acid has been sufficiently reduced, the putrefyers can continue the process of putrefaction since their spores are not killed by the acid. Thus by the combined action of the souring and putrefactive bacteria and a mold, both the carbohydrates and the proteins of the milk may be completely decomposed, the carbon all being liberated, as CO_2.

The carbon cycle may be still longer. The sugar in the plant may be eaten by an animal, and this animal by another, so that a given particle of carbon may enter into hundreds of combinations, become a part of many organisms successively, and continue these transformations for a very long period of time before it is returned to the air as CO_2.

The nitrogen cycle. Nitrogen, like carbon, is one of the chemical elements necessary to all forms of life, being a constant constituent of the living protoplasm. Animals are dependent for their supply of nitrogen upon the proteins in their food, and these proteins are derived directly or indirectly from plants. Plants generally are able to synthesize proteins, starting the **nitrogen cycle** with **nitrates.** The amount of nitrate present at any given time, even in the most fertile soil, is very limited. Therefore, the growth and yield of crops is dependent upon a continuous renewal of the supply of nitrates in the soil.

The first step in the nitrogen cycle is the formation of **amino acids.** Sugars are easily oxidized in the plant to various organic acids, and these are made into corresponding amino acids by the introduction of the amino group, or amino radical, (NH_2), which takes the place of one hydrogen atom in the molecule of the organic acid. Numerous molecules of amino acids combine together to make **proteins,** much as the simpler sugars combine to form the more complex carbohydrates. The proteins, as food, by a process of assimilation, become a part of the plant protoplasm. If an animal eats the plant, it digests the plant proteins to amino acids, and out of these reconstructs or synthesizes its own proteins and nourishes its protoplasm. During life the animal constantly excretes nitrogenous waste substances, largely as **urea** and **uric acid.** These wastes, as well as the proteins of dead plant and animal bodies readily undergo **decay** or **putrefaction.** This decay is primarily the work of the putrefactive bacteria, and the end product of decay of the nitrogen compounds is **ammonia,** (NH_3). The **nitrifying bacteria** now oxidize the ammonia to nitrates in two steps. One species of nitrifying bacteria oxidizes the ammonia to **nitrites,** and another species continues the process, forming **nitrates.** Thus the limited supply of the latter in the soil is constantly replenished.

Two other groups of bacteria have an important relation to the nitrogen cycle. Many kinds of bacteria when grown under conditions giving an inadequate supply of oxygen, that is, under anaerobic conditions, attack nitrates or decompose organic matter in such a way as to liberate the nitrogen as free nitrogen gas, which is then lost to the nitrogen cycle. These bacteria are called **denitrifyers** and the process, **denitrification.**

Serving to balance the loss of nitrogen from the nitrogen cycle by denitrification, there is another group of bacteria called the **nitrogen-fixing bacteria,** which are able to take free nitrogen gas from the air and "fix" or combine it with other elements to form proteins, and thus build up their own protoplasm. This capacity is not found among plants in general; therefore, these bacteria are of very great importance, as they make available for living beings the enormous supply of nitrogen in the air. Air is nearly four-fifths nitrogen.

Some of the nitrogen fixers are found in practically all soils that are not too acid or too alkaline. Several species live free in the soil, that is, not associated with any other organisms; and at least one species lives associated with leguminous plants, peas, beans, clovers, alfalfa, etc. Its presence causes those plants to develope tubercles or nodules upon their roots. In these nodules the bacteria are able to "fix" or combine the free nitrogen, more abundantly than when they live free in the soil. The clover or other legume furnishes a suitable carbon compound, probably sugar, to the bacteria, which utilize the sugar partly to furnish carbon, hydrogen, and oxygen for the synthesis of proteins, and partly to furnish the energy necessary in the process. On the other hand, the clover derives direct benefit from the nitrogen combined by the bacteria in its nodules, since in a soil with an inadequate supply of nitrogen, clover with nodules always makes a greater growth than without them. In order that the crop following the clover may have the full benefit of the nitrogen fixed by the nitrogen-fixing bacteria in the nodules, the clover crop must be turned under the soil and its nitrogen compounds must undergo decay and nitrification.

It will be seen that the nitrogen cycle is much more complicated than the carbon cycle, and that the former is very closely associated with the fertility of the soil. A fertile soil must have an adequate supply of organic matter, and must be well aerated by stirring or cultivation to make the conditions favorable for the growth and activity of the putrefactive, the nitrifying, and the nitrogen-fixing bacteria. Also it must not be too acid, since the bacteria are generally more sensitive to acidity than the crops for the benefit of which their work is necessary.

CYANOPHYCEAE. Blue Green Algae.

This class of Algae is distinguished by the possession of a bluish-green pigment, **Phycocyanin,** in addition to the chlorophyll, giving them a bluish-green rather than a grass green color. They have no definite chloroplasts, the pigments being distributed throughout the cytoplasm. They also have no definite nucleus. Their reproduction is by **cell division** alone for many; others reproduce by fragmentation of filaments, the fragments being called **hormogonia**; some form asexual spores. No sexual reproduction is known among them.

Certain species of Cyanophyceae give bad odors or tastes to water supplies, in which they grow. They may be exterminated by treating the water with copper sulphate, at a concentration which is fatal to the algae, but not poisonous to animal life.

Some blue greens are agents in the deposition of **marl,** a lime deposit of fresh-water lakes, and found in Michigan and other parts of the world. Marl is one of the raw materials out of which cement is manufactured. It may be consolidated into limestone rock.

A. Study several forms of blue-green algae which show increasing complexity of structure from unicellular or globular masses of cells to simple filaments with no differentiation of cells, and filaments having differentiated cells. Draw each species studied.

1. **Gloeocapsa** or similar species. How does the mass look to the naked eye? What is the shape of individual cells? The shape of the colony if there is a colony? How is the colony formed? By what is it held together? Is there a definite chloroplast?

2. **Oscillatoria,** a filamentous form with motion. What is the appearance to the naked eye? What is the size and shape of the filaments? Of individual cells? Are the cells all alike? Note three kinds of motion. What are they? Can you recognize a chloroplast? A nucleus?

3. **Nostoc.** What is the shape and appearance of the mass of plants? Under the microscope, how many kinds of cells are there? How are they arranged? The few differentiated

cells are called heterocysts. Their use is uncertain. The absence of a definite nucleus and of a definite chloroplast, as well as the simple structure of the Cyanophyceae, all mark them as the most primitive class of chlorophyll-bearing plants. Considering Gloeocapsa, Oscillatoria and Nostoc as a possible evolutionary line, notice how little change is necessary in Gloeocapsa to produce Oscillatoria, and in the latter to produce Nostoc.

CHLOROPHYCEAE. Green Algae.

In this class of algae the chlorophyll is not generally associated with nor masked by another pigment as in the other classes of Algae, hence they are of a grass green color. They vary in complexity of structure from single cells to unbranched and branched filaments. Very few forms are more than a few inches long at the largest. Some reproduce only by cell division, but most of them have **asexual** or **sexual spores** or both. A complete classification of an alga is given as an example. The distinguishing characters of each category, or classification group, are given. **Classes** are divided into **Orders,** and one of the orders of the Chlorophyceac is the

ORDER CONJUGALES.

Plants of this order are either unicellular or of unbranched filaments. The former reproduce by cell division, but all reproduce sexually by the union of two similar gametes which are the protoplasts, or entire protoplasmic masses, of two vegetative cells. The union of similar gametes, however they may be formed, is called conjugation; hence the name of the order. One family of this order is the

FAMILY ZYGNEMACEAE.

The plants of this family are composed of cells arranged in unbranched filaments. They have peculiarly-shaped chloroplasts, one genus having them in the shape of a rectangle. Another, Zygnema, for which the family is named, has two star-shaped chloroplasts in each cell. A third genus has chloroplasts in the shape of spiral bands or ribbons.

GENUS SPIROGYRA.

A. Examine fresh material with the naked eye. Note the general appearance, color, feeling, filamentous habit.

B. Mount a small quantity in water, taking pains to float the filaments apart from one another before covering. Do not mount a dense mat.

1. Examine under the low power of the microscope and note the form of the filament as a whole, and the shape and arrangement of the cells which compose it. Are the end cells of the same form as the others? Are the cells of uniform length and diameter in the same filament? In different filaments? How long is one cell compared to its width? Estimate the diameter of your field under high power at about ¼ of a millimeter if it has not been measured, and under low power at 1½ millimeters. How long is the whole filament? How long is the average cell?

2. The green bodies within the cells are the **chloroplasts.** What shape and position have they? How many are there in each cell? Is the number variable in cells of the same filament? In cells of different filaments? On the basis of differences in cell size and shape, and of the number of chloroplasts, can you find more than one kind of Spirogyra in your mount? If so, how many? Ask the instructor if they are different species.

3. Within the chloroplasts are numerous highly refractive bodies called **pyrenoids.** Watch for any change in their appearance which may occur when a drop of iodine solution is added at the edge of the cover glass. Is starch present? If so, where?

4. Locate the nucleus, suspended by strands of cytoplasm.

5. Draw a single cell, showing all the structures which you can find. Also diagram the cell as it would appear in median optical section. Compare carefully with the other plant cells which you have studied, particularly with regard to cytoplasm, nucleus, and vacuole.

C. **Sexual Reproduction.** Make a mount of material showing the process of **conjugation.** Look for parallel filaments connected by conjugation tubes.

1. Find and draw, if possible, a case where the cells have joined, but each contains its own protoplast.

2. Find a case where the protoplast is passing over into the other cell. Draw.

3. Find and draw a case where the two conjugating cells have completely united, the contents of the two cells having fused into a **zygospore.**

One of the common species of Spirogyra has the following name and characteristics:

Spirogyra Weberi. Filaments 22-28 microns in diameter, cells 6-16 diameters long; chloroplast single, slender and loose, with large pyrenoids, making 3½-6 turns in the cell; fertile cells not swollen, or only enough to contain the spores, which are ovoid, 1½-2 diameters long, diameter of spore 26-30 microns.

Spirogyra orthospira is another species. Filaments 60-66 microns in diameter, cells 2-3 diameters long; chloroplasts 7 or 8, very slender, pale green, usually straight and parallel, sometimes inclined, making ¼ turn in the cell; fertile cells little or not at all swollen; spores at maturity brown, lenticular, about 72x48 microns.

Write a description of the species you studied, following the above descriptions as models. This is a typical description of a species as used in systematic botany since the time of Linnaeus.

An outline of the complete classification of Spirogyra is:

Kingdom, Plantae.
  Division, Thallophyta.
    Sub-division, Algae.
      Class, Chlorophyceae.
        Order, Conjugales.
          Family, Zygnemaceae.
            Genus, Spirogyra.
              Species, Spirogyra orthospira.

DIATOMS.

The plants known as **diatoms** are sometimes classified as a family under the order **Conjugales** but are perhaps better ranked as a separate class of algae. They are of interest from several points of view. They are mostly unicellular, chlorophyll-bearing organisms, sometimes in filaments, with two characteristics more commonly found among animals, the presence of a firm shell, and locomotion, which many of them show. The shell is made of silica, a chemical substance familiar in the form of ordinary white sand, and it is transparent, like glass. It is very indestructible, and the accumulation of these shells, on the bottom of waters where diatoms are abundant leads to the formation of **diatomaceous earth.** Beds of such earth are known that are hundreds and even several thousands of feet thick. Diatomaceous earth has several economic uses. One use is as polishing powder. Another is as an **absorbent of nitroglycerine** in the manufacture of **dynamite.** The latter is less likely to explode than is the nitroglycerine. Diatomaceous earth is also used in the manufacture of **sound-proof material.** Some species of diatoms give bad tastes or odors to water supplies.

Diatoms are very widely distributed, in both fresh and salt water. In the polar regions of the ocean they are among the most numerous constituents of the **plankton** or floating life, and are the chief food of many small animals upon which fish and larger animals feed. For general biology, therefore, they are of great importance. The beauty and variety of structure shown in the architecture and ornamentation of their shells has made them special objects of scientific curiosity, and about 10,000 species have been described:

A. Vegetative structure of cells. Find and draw sever forms of diatoms and note their slow **locomotion.**

1. Note the shell, composed of two parts fitting togeth like a **pill-box and its lid.** These shells are usually beautifull marked. Show some of the markings on one.

2. The **chloroplasts.** The green color is masked by a brown pigment which gives the chloroplasts a brownish green color. There may be several simple chloroplasts or one. Which

is it in your specimens? Hydrochloric acid will remove the brown color, leaving the clear green of the chloroplasts visible.

3. Often one or more definite **oil drops** are visible in the cell.

4. If you are fortunate, you may find a cell in process of division. The protoplasm divides into two portions and the two halves of the shell separate, each half forming a new half-shell whose edge is overlapped by the old half. This method of division always results in two cells one of which is the same size as the parent cell, and the other is smaller. When the size becomes too small, the protoplasm throws off both halves of the shell, and grows to a larger size, either alone or after uniting, by conjugation, with another similar cell which has discarded its shell. You are not likely to see this latter process.

VAUCHERIA.

Vaucheria belongs to the same class as Spirogyra, but to a different order and family. It is studied as a representative of those green algae which reproduce asexually by swimming spores called **zoospores,** and sexually by **gametes** differentiated into male and female, and each gamete produced in a separate organ.

A. With the naked eye, study the fresh plant growing on earth or submerged in water. Note its color, its general appearance, and the flannel or felt-like feeling when taken between the fingers.

B. Take up a small portion of the growth, separating the filaments carefully on the slide in water, cover, and study under the microscope. Describe the following structures:

1. The **filament as a whole.** Compare it to that of Spirogyra. Does it have a wall, protoplasm, and chlorophyll? Is it divided into cells as was Spirogyra? How does its length compare with its width? Is it branched?

2. Under high magnification make out the layer of **cyto-**

plasm lining the cell wall and containing the **chloroplasts.** How thick is this layer? What fills the center of the chamber within this layer?

3. What is the shape and arrangement of the chloroplasts? Are they in more than one layer? Give a reason for your answer. Are pyrenoids present? Note colorless, shiny globules among the chloroplasts in some filaments. Do they color blue-black with iodine? They are **oil.** What other plant that you studied stored reserve food as oil instead of starch?

4. In filaments with the chloroplasts widely separated, you may be able to find **nuclei.** They will be difficult to find, but are globular in shape, smaller than the average chloroplast in diameter, and with a very small **granule** in or near the center. You may find several near each other, and see them moving along between the chloroplasts.

Draw a filament with low and high magnification, showing all the structures as seen in a surface view and draw another in optical section of the filament.

C. **Asexual reproduction.** In the fresh material you may study the method of asexual reproduction. This is by **swarm spores,** which are motile spores, and often called **zoospores.** In the ends of certain branches you will find the tip somewhat swollen, and of a much darker green color than the rest of the filament. Why is it darker? This tip is cut off by a wall. Then the protoplasmic contents escape as a large ovoid, free-swimming body, covered all over its surface with numerous short **cilia.** It is at first without a cell wall. After it swims about for a time it comes to rest, becomes more spherical, then secretes a cellulose membrane on its surface, and grows out into a new filament. This is the type of an asexual spore which, without uniting with any other cell, reproduces the species.

D. **Sexual reproduction.** In this method of reproduction in Vaucheria, not only do two cells unite to form a spore, but the cells that unite, the gametes, are not alike, and are produced in different organs, though on the same plant.

Growing out of the side of a main filament or on a short side branch, find two organs, a hook-shaped, slender **antheridium** and an ovoid **oogonium.** How are they situated in relation to each other? The antheridium produces many small, motile sperms or male gametes, and the oogonium produces one large, non-motile egg or ovum, the female gamete. When the motile male gamete unites with the female gamete or egg, the process is called fertilization, or it is **heterogamy.** The spore thus formed is called an **oospore.** Note the very thick wall around the oospore, inside of the oogonium wall. Like the zygospore of Spirogyra the oospore of Vaucheria can go through a resting period, through winter or a dry period, and grow into a new plant when the conditions again become favorable.

PHYCOMYCETES. Alga-like Fungi.

This is one of the classes of **fungi.** The name is given because in certain characteristics these fungi are like some of the algae. They are more complex than the bacteria. The thallus or body of the plant is generally a much-branched, filamentous structure called the **mycelium.** Reproduction is by both asexual and sexual spores. Asexual reproduction is commonly by means of **zoospores**; or by **conidia,** spores formed on the ends of special branches called **conidiophores**; or by spores formed in large numbers within spore-cases called **sporangia,** which are borne on stalks called **sporangiophores.** Sexual reproduction is by either **zygospores** or **oospores.**

The Phycomycetes are of great importance economically, since many of the species are parasitic upon either plants or animals, some on fish, and numerous ones on crops of field or orchard, producing blight, rot, or wilt of plants.

RHIZOPUS NIGRICANS. Black Bread-Mold.

This is a common **saprophytic** fungus, reproducing sexually by the **union** of nearly **similar gametes** and asexually by **spores** produced in **sporangia** or spore-cases.

A. Examine the material with the naked eye and note its general appearance and its relation to the substratum. Can you distinguish the groups of erect branches, sporangiophores, with the swollen white or black tips, the sporangia? Are these groups of sporangiophores attached to the substratum? Is there any connection between neighboring groups?

B. Mount a small quantity of the mold and examine for vegetative filaments. The individual filaments of which fungi are composed are called **hyphae.** These taken collectively constitute the **mycelium.** Note whether cross walls are present. Can you distinguish any nuclei? When the protoplasm of a plant contains many nuclei not separated from each other by walls, the plant is called a coenocyte. What alga studied was coenocytic?

C. **Asexual reproduction.**

1. Examine under the low power and draw a single group of sporangiophores, showing the hypha connecting one cluster of sporangiophores with another cluster, and called the **stolon,** and the hyphae modified to serve as holdfasts, root-like branches.

2. Find and draw under the high power a series of at least three sporangia, illustrating the development of the following structures. Note the **sporangium wall.** Look for the **columella,** a wall separating the sporangium from the sporangiophore. What is its shape? Draw a columella with adhering spores, after the rupture of the sporangium wall. Examine some of these asexual spores and note their color, size, shape and markings of the membrane. Draw several spores showing any variation you may discover.

3. From the instructor obtain a mount showing germinating spores. Draw two or three of various ages showing the young mycelium.

D. **Sexual reproduction.**

1. Sexual reproduction occurs in Rhizopus only when both **male and female mycelia** are present. Examine dishes in which both male and female mycelia have been planted. Along the zone of contact of the two mycelia, note the dark region in which **zygospores** are being produced. Make a mount of material from this region. Look for club-shaped, modified hyphae which meet in pairs. If the male and female plants are not available, use mounted slides showing the formation of the zygospores. The two hyphae of each pair arise respectively from the male and female mycelia. At the end of each hypha a terminal portion is cut off, containing the **coenogamete.** The wall between the two adjoining **coenogametes** dissolves and they unite to form a **zygospore,** which becomes surrounded by a heavy wall. What is the nature of its surface? Draw a mature zygospore and several earlier stages in the process of sexual reproduction. The cells from which the coenogametes were separated remain attached to the zygospore and are called **suspensors.**

ASCOMYCETES. Sac Fungi.

In this class of fungi certain spores are formed in sacs called **asci,** and there are usually just 8 spores to one **ascus.** The spores are called **ascospores.** In most of the species the asci are not borne separately, but are found in small or large numbers in special fruiting bodies called **ascocarps,** which are of many sizes, shapes, and colors. Some are microscopic and are buried in the tissue of leaves or twigs. Others are visible to the naked eye as small black specks on the surface of leaves. These are closed or have small openings, and are called **perithecia.** Others are still larger, and more or less widely open, like trumpets or cups or saucers, and these open ascocarps are called **apothecia.** Larger than these are the ascocarps of the **morel,** an edible fungus, called a mushroom, whose fruiting body is several inches tall. It consists of a nearly **cylindrical stalk,** bearing a conical, brownish, corrugated, sponge-like **cap.** The whole surface of the cap is covered by a continuous layer of asci, each ascus containing 8 ascospores. The **truffle** is another ascomycete whose ascocarps are edible, but they mature under ground, and are collected by the use of trained dogs or pigs, which locate the truffle by the scent. It is considered a great delicacy in France.

Many of the Ascomycetes are parasites, causing blight, rot, or mildew· One, called ergot, a parasite on rye, is used in medicine. The botanical name of this fungus is **Claviceps purpurea.**

UNCINULA SALICIS. Willow Mildew.

A parasitic fungus, living on the surface of its host, on which it appears as a whitish coating over the leaf or in small spots, with dark specks just visible to the naked eye, scattered over the spots when it is mature. The willow mildew, **Uncinula salicis**, the lilac mildew, **Microsphaera alni** or other species may serve for study.

A. Note the appearance of the parasite on the host, its color, distribution, size of spots, presence or absence of black specks. Draw the specimen, showing the points noted.

B. Reproduction by **conidia.** This study is most satisfactorily made from freshly gathered material. With a scalpel or similar instrument scrape some of the powdery growth from the surface of the host and mount in water. Under high power, look for the reproductive bodies called **conidia.** They are single cells, and are formed in chains by successive cell divisions on a branch from the mycelium called the **conidiophore.** Draw and describe the conidiophore and conidia. Show the mode of origin of the latter, and the relation of the former to the mycelium. The conidia are produced in great numbers, are blown by the wind from plant to plant, and hence are a means of rapid spread of the parasite during the growing season. If material is not available for this study, read carefully the life history of mildew in your text-book and get a clear idea of the formation of conidia and the part they play in the life history of the fungus parasite.

C. **Perithecia.** The black specks scattered over the mildew spots on a leaf are called perithecia. (Singular, perithecium). Scrape some from the leaf and mount for study. Under low power note the size, shape, and color of the perithecia, and the occurrence of outgrowths, or **appendages,** on them. These appendages are used as aids in distinguishing genera of the mildew family. Note especially their length and the character of their unbroken tips. **Uncinula** has **hooked tips. Microsphaera** has **tips dichotomously branched.** Draw a mature perithecium with its appendages.

Asci and **Ascospores.** Select a mature perithecium and watch it in the microscope under low power while you press on the cover glass with the tip of a needle directly over the perithecium, bursting it. The colorless sacs that escape are the **asci** and they contain **ascospores.** What shape are the asci? How many in a perithecium? Count them from several perithecia and compare your results with those of your neighbors, getting the range of variation. What is the color, size, and shape of the ascospores? How many in an ascus? Get the range of variation here also. Draw one or more asci showing the shape and ascospores within it.

Perithecia containing asci and ascospores live through the winter and are the means by which the fungus survives a season unfavorable to growth.

BASIDIOMYCETES.

The fungi of this class have a filamentous, multicellular mycelium which may be parasitic in the leaves, stems, and fruits of many herbaceous plants as are the **rusts and smuts;** it may be saprophytic in soil, as are many of the **mushrooms** and **puffballs;** or it may be able to destroy the woody tissue of trees as so many of the **bracket fungi** do. The brackets, mushrooms, puffballs, etc. are merely the fruiting bodies or spore-bearing structures, often called **sporophores.** The spores are borne commonly in clusters of 4 on a basidium. These basidia are usually club-shaped, bearing the spores at the thicker end, each spore on a very short and slender stalk. The spores are called **basidiospores.** In the rust fungi and the smuts the basidia are separate and not collected into a definite sporophore. In those with a definite sporophore the basidia form continuous layers covering certain portions, as the surface of the **gills** of mushrooms, or lining the internal cavities of puffballs.

PUCCINIA GRAMINIS. Wheat Rust.

Puccinia graminis, a fungus causing the disease of wheat known as rust, is a parasitic fungus reproducing asexually by various types of spores, and living alternately on two different hosts, 1, a grass, often wheat, and 2, the barberry.

A. The **phase on wheat.**

Examine a portion of a wheat plant infected with rust. Note the numerous dark areas, **sori** (singular **sorus**), in which the fungus occurs. Make a sketch showing the parts of the plant on which they are found. What is their position with regard to veins? Use a hand lens and the low power of the microscope to see if the sorus causes a rupture of the epidermis.

Scoop out the content of a sorus with a needle or the tip of a scalpel, tease out in a drop of water, and examine under high power. You will find at least one kind of spores, and probably two:

1. The **one-celled uredospores,** sometimes called summer spores, because they are the chief means of distributing the fungus from one wheat plant to another during the summer. What is their shape? Color? Have they a stalk? Of how many layers is the wall composed? Has it any markings? Can you make out germ pits in the inner wall about the equator? Make a careful drawing.

2. The **two-celled teleutospores,** sometimes called winter spores, because they live through the winter, reproducing the fungus in the spring. Compare in all respects with the uredospore and draw.

B. The **phase on barberry. Aecidium stage.** When the teleutospores germinate in the spring, a chain of four cells is formed called a **basidium,** each cell of which bears a single **basidiospore** at the apex of a slender branch. These basidiospores germinate on barberry leaves, producing in the tissues of the host an extensive mycelium, from which ultimately develop the **aecidia** on the lower surface of the leaves and the **spermogonia** on the upper surface.

1. Examine and draw a diseased barberry leaf. Note the size, shape, and color of the infected spots (sori). Scattered over the surface of the sorus you will see small openings. These are the openings of the aecidia, from which the aecidiospores escape and are scattered by the wind. Draw a leaf showing a sorus; and an enlarged view of the sorus, showing the openings into the aecidia.

2. Study prepared cross-sections of the barberry leaf.

a. Examine under low power, and describe the differences between the affected and unaffected parts of the leaf. Make an outline drawing of the whole section of the leaf and aecidia.

b. Study the aecidium under high power, and make a detailed drawing showing the wall, the **peridium,** the chains of **aecidiospores,** the **hymenium** (a layer of hyphae bearing the chains of aecidiospores at their ends), and the **mycelium** from which the hymenium arises.

c. Under high power, examine the flask-shaped spermogonia, which generally occur on the opposite side of the leaf from the aecidia. Draw, showing the openings to the surface and the slender filaments forming spermatia by successive abstrictions. These appear to have no function at present.

AGARICUS CAMPESTRIS. The Common Mushroom.

Agaricus campestris is a saprophytic fungus whose mycelium grows for several weeks or longer in soil rich in organic matter, and whose fruiting body or **sporophore,** grows up in a night, producing and scattering the spores.

A. The **Sporophore.** What is its size and its shape as a whole? The two main portions are the stalk or **stipe** and the cap or **pileus.**

1. The **stipe.** What is its shape, its length, its diameter, its color? How is it attached to the pileus? Does it have a ring or **annulus** on it? If so, where?

2. The **pileus.** What is its size, its shape, its color?

3. The **gills.** On the lower side of the pileus find the thin plates called gills. How are they arranged in relation to the pileus and to the stipe? What is their color? Are all of equal length? If not, what different lengths are there? The gills are the specific spore-bearing organs.

4. **Spore-print.** Note how it was made and the color of the spores in mass. The color of spores is used in the classification of mushrooms, and spores are not always the color of the gills to the eye, though usually they are.

5. Make a microscopic study of the gill structure, from slides of the mushroom **Coprinus,** showing cross section of the gills. The surfaces of the gills are made up of club-shaped cells called **basidia** which bear the spores. Note the arrangement and proportions of the basidia. How are the spores attached to a basidium?

6. **Spores.** In the preparation of the gill or in a new one made by scraping a fresh piece of gill in a drop of water, study the spores under the microscope. Are they of the same color as when seen in mass? If not, what makes the difference? What shape are they? What interior structure do they show? What peculiar markings, if any, do the spore walls exhibit? Ask what these spores develop into when they germinate, and make a note of it.

7. **The veil** or **velum.** Note the difference between a young and an old sporophore, the former with the velum unbroken. Where is it situated? What purpose does it serve? What becomes of it as the sporophore grows older?

Write an account of the life history of Agaricus, and make the following drawings: a side view of the whole sporophore; the cut face of one split into halves through stipe and pileus; the lower surface of the pileus showing the gills; one or more basidia showing spores attached; several spores showing range of size and shape.

FOMES APPLANATUS. A Bracket Fungus.

Shelf-like or bracket-like fungi are often found on the trunks of trees or on fallen logs. Most of them are saprophytes but a few of them are parasites. **Fomes applanatus** is often found growing from wounds or dead spots on live trees and it is believed to be parasitic to some extent. In the family to which it belongs the basidia on which the spores are produced form a lining inside of cylindrical holes or pores, opening on the lower side of the fruiting body or sporophore. The mycelium lives in and destroys the wood.

Describe the sporophore and make three drawings of it, a top view, a bottom view and a vertical section.

LICHENS.

Lichens are combinations of two organisms, a **fungus** and an **alga,** which grow as if they were one. They grow on earth, rocks, tree trunks, etc. Some are thin **crusts**; others **leaf-like,** growing flat on the substratum; or they hang in festoons from the twigs and branches; or they are like shrubs, growing on trunk or twigs or upon earth. They have a distinctive grey-green color, the green due to the alga, the grey to the fungus enwrapping the alga cells. The latter are usually green or blue-green algae, and the fungi are mostly Ascomycetes, but a few are Basidiomycetes. Each organism reproduces itself independently, and the species of algae can lead a separate existence while the fungus can not. Several of the lichens are edible either by men or animals. One, **Roccella tinctoria,** is the source of **litmus,** a coloring matter used in chemistry. One, the so-called **reindeer moss, Cladonia rangiferina,** is an important source of food in winter for reindeer in the arctic regions. "Iceland Moss" is used in Iceland both as a food and a medicine. It is called **Cetraria Islandica.** Another, **Lecanora esculenta,** grows on the ground in deserts from the Sahara to Central Asia, and was probably the **manna** which fed the Israelites in the wilderness.

A. Observe several species of lichens furnished, describing their size, shape, and relation to the substratum. The reindeer moss is common even in Michigan.

B. Tease out a small portion of a thallus into exceedingly fine fragments and examine with the microscope. Find the **alga cells,** which will probably show as **single cells or small** cell masses. Notice the **fungus filaments in contact with or** enclosing the alga cells.

Draw a lichen natural size, and also the **alga cells and** fungus fragments seen in the microscope.

C. Note some specimens which show the very small **cups** or **apothecia,** which are the fruiting bodies of the fungus only. Sections through the cups, or fragments of the inner surface teased out, will show a layer of asci with ascospores, similar to those of other Ascomycetes.

Draw a fragment to show these.

Lichens may also be reproduced vegetatively, by small fragments consisting of both fungus and alga. Such fragments may be blown about by the wind and grow wherever they find a suitable situation.

BRYOPHYTA. Mosses and Liverworts.

This is the division of the plant kingdom next higher than the Thallophyta in complexity of structure and reproduction, though none of them is as large as the largest thallophytes. They do not rise more than a few inches above the ground, though in water some mosses may extend several feet, or build up a mat several feet thick in bogs.

None of the Bryophyta have vascular bundles, and this is perhaps the reason why they do not rise far above the surface of the earth.

In reproduction they have a definite **alternation of generations,** a **sexual generation** or individual, called the **gametophyte** and reproducing by **male and female gametes,** alternating with an **asexual generation** or individual, called the **sporophyte,** and reproducing by **asexual spores,** but not by gametes.

The two generations are not equally independent. The gametophyte is the independent plant. It is attached to the earth by rhizoids, and never has true roots. The sporophyte is dependent upon the gametophyte and is attached to it.

HEPATICAE. Liverworts.

In this class of plants there are some whose plant body is a thallus, and others in which there is a differentiation into stem and leaves, but the leaves are only one cell thick, and without ribs of any kind. They are attached to the earth by rhizoids, which differ from true roots in being single cells or single rows of cells, are without a root cap; and have an origin at the surface of the part of the plant from which they arise. Liverworts not only show the transition **from the thallus form** of plant body **to the leafy stem form,** but they also show transition from **aquatic** life, or life in the water, to **terrestrial** life, or life upon the more or less dry land.

A. Draw and describe each of the following liverworts. Give size, shape, and mode of branching of each, and note whether it is thalloid in form, and whether aquatic or terrestrial:

1. Riccia fluitans;
2. Riccia natans;
3. Porella platyphylla.

One species may be studied more in detail.

B. **Marchantia polymorpha. Gametophyte.** Note size, shape and position of the thallus on the earth, also its branching. At first the two branches are equal in size, and the branching is dichotomous. A little later one branch ceases growth, the other continues growth. At each forking, the branch remaining short is on the opposite side from that of the last preceding branch, hence the short branches are alternately right and left. The thallus is attached to the soil by slender fibrous rhizoids. With a hand lens note the surface of the thallus divided into diamond-shaped or rhombic areas, each with a stoma at the center. These stomata open into air spaces in which many chlorophyll-bearing cells are situated. Notice the small cups on the upper surface, filled with small green bodies, the gemmae. These are vegetative reproductive bodies, a kind of bud, by which the thallus reproduces itself directly, and without alternation of generations.

Marchantia also reproduces sexually, and the male and female sexual organs are borne on separate plants.

1. Draw the male and female plants of Marchantia showing the special branches of the thallus for bearing the reproductive organs. Those on the male plant, the **antheridiophores,** bear ovoid **antheridia** sunken into the upper surface of the star-shaped receptacle, while those on the female plant, the **archegoniophore,** bear the flask-shaped **archegonia,** on the lower side of a receptacle shaped like an umbrella frame. The male and female plants with their reproductive organs constitute the sexual generation or gametophyte. The fertilized egg develops into the asexual generation, or sporophyte, which is only one or two millimeters long at the largest, and matures in place on the lower side of the receptacle of the archegoniophore.

2. With prepared slides, describe and draw an **antheridium,** and an **archegonium.** The former produces thousands of sperms, the latter one egg each. The antheridium is ovoid, with a wall and a stalk. The archegonium has a bulb-shaped venter in which is the egg, and a long neck, in which a **canal** forms the path of the **sperm** or male gamete to the **egg** or female gamete.

C. **Sporophyte.** The sporophyte is the generation alternating with the gametophyte. Its existence starts with the growth and nuclear and cell division in the fertilized egg. Later its tissues differentiate into three main organs, the **foot** which serves as an organ of attachment, of absorption, and to a certain extent of digestion; the **stalk,** which conducts food and water from the foot to the capsule containing the spores, and which finally lengthens and pushes the spore case out of the protecting envelopes when the spores are mature; the **capsule** in which the spores and elaters are developed.

1. Tease out from the underside of the receptacle of the archegoniophore both mature and immature sporophytes. Note that they are attached to the receptacle where the archegonia were situated. The fertilized egg did not escape from the archegonium but developed in the place where fertilization occurred. Also note that the individual or generation

that developed from the fertilized egg is entirely unlike the plant which bore the sexual organs and gametes. This is what makes the life history an alternation of generations.

Draw and describe the sporophyte with its **foot, stalk, capsule,** and two **protecting envelopes.** The inner one is the remains of the archegonium wall; the outer one grew like a sac around the archegonium, starting before fertilization.

2. Tease out the contents of a mature capsule and of another not quite mature. You may find them both on the same receptacle. From the mature one you will get spores and elaters. Note the size, shape, and cell contents of the spores. When these undergo further development they produce the gametophyte, again alternating the generations.

The long slender structures, the **elaters,** when mature, aid in distributing the spores gradually, rather than allowing them to fall in a heap when the capsule opens. They cling together, holding the spores between them, twisting by **hygroscopic action** as the moisture in the air varies from moment to moment, with the result that the spores are sifted out very gradually and widely distributed by the wind. Draw a spore and an elater.

3. From the capsule not quite mature find **spore mother cells** in the process of division. Each spore mother cell divides into four spores. You should find the stage of development before the 4 spores have separated. Note that the walls of the spores are so placed that each spore is a **tetrahedron** in shape, except that its one outer face is curved instead of flat.

The fact that these spores are **formed by division** of a spore mother cell and not by a union of gametes, shows them to be **asexual spores,** and the generation that produces them, the **sporophyte,** is the **asexual generation.**

Draw a spore mother cell showing the tetrahedral division.

4. **The Reduction Division.** The first nuclear division that takes place when the spore mother cell divides to form the asexual spores, is different from all the other nuclear divisions in the life history of the plant. It is the so-called **reduction division.** When the gametes, the egg and the sperm, unite in sexual reproduction, each gamete, however different

from the other in size, shape, and motility, is equal to that other in that it has a nucleus with the same number of chromosomes. This number is also the same as that in the nuclei of all the cells in the gametophyte or sexual generation, and is indicated by the letter x. After the union of the gametes the fertilized egg, or **zygote,** as it is technically called, has 2x chromosomes. This double number is found in the nuclei of all the cells of the sporophyte, including the original nucleus of the spore mother cell; but **in the reduction division the number of chromosomes is reduced from 2x to x.** Hence each spore as well as every cell in the gametophyte that develops from it contains x chromosomes in its nucleus.

Not only does the reduction division halve the number of chromosomes in the nucleus but the method by which it occurs has another effect of great importance to the process of heredity, for in this same division there regularly occurs a new distribution of paternal and maternal chromosomes, involving new combinations of hereditary characteristics to be passed on to the next generation.

The reduction division is important, therefore, in that it halves the number of chromosomes and makes possible new combinations of hereditary characters. In all the plants of the three higher divisions of the plant kingdom, the Bryophyta, Pteridophyta and Spermatophyta, it takes place in the division of the spore mother cell when the asexual spores are formed.

MUSCI. Mosses.

The true mosses constitute the second class of the Bryophyta. They are very numerous as individuals and as species, rarely over an inch or two high, forming dense mats of green, in forests, on bare soil, or even on the bare surfaces of rocks, and in bogs, swamps or streams. Mosses grow from the tropics to the arctic regions. In the polar regions, mosses and lichens form a large proportion of the vegetation. Mosses all have a leafy stem belonging to the gametophyte generation, and it is attached to the earth by rhizoids. There are no true roots but the rhizoids are more than one cell long. Besides the leafy stem the gametophyte generation includes a branching filamentous structure, called the **protonema,** which much resembles an alga in appearance. The protonema develops from asexual spores, and on it the leafy moss plants arise as vegetative buds. Many mosses also produce a secondary protonema vegetatively from leaf or stem, and by this means, and some other vegetative methods of reproduction, mosses may multiply and extend their mats without reproducing sexually. However, some annually, others more rarely, reproduce sexually, with alternation of generations. The **sporophyte** is larger and more complex in structure than that of the liverworts, varying from a few millimeters to 5 or 10 centimeters in length. It does not have a separate existence from the gametophyte, but manufactures part of its own food in chlorophyll-bearing tissue. The wide distribution of mosses and the ability of some to grow in situations where few other plants can survive, give them an importance in the economy of nature greater than their small size would lead one to expect.

Some species growing in bogs are important plants concerned in the accumulation of peat, which consists of plant structures only partially decayed. The bacteria and fungi that quickly decay plant remains in other situations, find in the bog, conditions unfavorable to their development. Hence decay is incomplete. Similar conditions existing in past geological time over considerable areas of the earth, probably account for the accumulation of plant remains which resulted in the formation of the coal beds. However, the plants that formed the bulk of the coal belonged, not to the true mosses, but chiefly to the fern-like plants.

A **Mnium.** 1. Observe the green filaments growing on earth, the **protonema,** and describe their appearance. Mount a few filaments and study under the microscope. Note color, structure, branching and cross walls. In what respects are these filaments like algae? Can you find buds on the protonema from which the leafy moss plants develop? Draw. The protonema is formed by the germination of the asexual spores, but protonemata can develop from any part of some moss plants.

2. Describe the general appearance of the **leafy moss plants** as they grow in mass, their height, their crowded growth. Is there evidence of their spreading by vegetative

reproduction? Separate a single plant, washing the earth from the lower part of it. Note the arrangement of the leaves on the stem and the rhizoids on the lower part of the plant. What is the shape, size, and color of the stem? How far down is it clothed with leaves? Mount a leaf for microscopic study. It is composed of **blade,** or **lamina,** and **midrib,** or **costa.** Describe the shape and structure of each and the shape and size of the cells in these two portions. Test for starch. Draw a moss plant four times natural size, a leaf in outline, and a few cells of the lamina to show shape and arrangement of cells.

a. **Male plant.** The **antheridia** are borne at the apex of the male plants. Tease out the tip of one plant, and examine under the low power for **antheridia** and **paraphyses.** Note the size and shape of the antheridia. Compare with the antheridia found in Vaucheria and in Marchantia. Draw an antheridium and a paraphysis.

b. **Female plant.** The female reproductive organs, like the male, are borne at the tip of the shoot. Compare the male and female plants with reference to the arrangement of the leaves at the tip. Tease out the tip of a female plant and examine with the compound microscope for the female reproductive organs. In the mosses as in the liverworts we find the egg cells borne in a **multicellular archegonium.** This is a more or less flask-shaped structure composed of a bulb-like portion called the venter, containing the egg and a long neck with a central row of canal cells. At the maturity of the archegonium these canal cells disintegrate, leaving a canal through which the sperm swims to the egg. Some archegonia are attached by a stalk. Is this the case in the moss? How does the moss archegonium differ from that of Marchantia? Paraphyses are found among the archegonia as well as among the antheridia. Draw a paraphysis and an archegonium showing all parts.

B. **Polytrichum** or other species with sporophytes.

The fertilized egg by cell division, growth, and differentiation develops into the **sporophyte** or **2x generation.** In the material furnished for study, the sporophyte is attached to the female gametophyte.

1. Note the parts of the sporophyte: a **sporecase** or **capsule, stalk,** and **foot.** The latter is embedded in the tip of the female plant. What are the size and shape of the stalk and capsule? Draw the sporophyte attached to the gametophyte. Note the lid, **operculum,** on the capsule. Remove it and look for a row of teeth, **the peristome.** Draw the peristome. Remove some **spores** from the capsule. What is their size, shape, and color? These are the asexual spores, the production of which marks the end of the 2x or sporophyte generation and the beginning of the x or gametophyte generation. The student should not fail to note that the spores belong to the gametophyte generation on the basis of the number of chromosomes in the nuclei. When the spores germinate they develop into protonemata and on these grow the leafy moss plants. These two phases together compose the sexual or gametophyte generation of the mosses, all of whose nuclei have x chromosomes.

PTERIDOPHYTA. Ferns and Fern Allies.

The plants in this division of the plant kingdom are the ferns and fern allies, though some of them do not look like ferns. They are alike however in having an alternation of generations with both generations living as independent plants, the **gametophyte,** being a very small **thallus** with **rhizoids,** and the **sporophyte** being much the larger, with true **roots, stems,** and **leaves.**

There are vascular bundles in the sporophytes and this enables them to reach a much larger size than any of the Bryophyta. Some tree ferns reach a height of 30 to 40 feet, and in past geological time some fern allies attained the height of about 100 feet. In reproduction they have a general similarity to the Bryophyta. The gametophytes bear archegonia and antheridia with eggs and sperms respectively, and the sporophytes bear asexual spores which develop into the thalloid gametophytes.

FILICINEAE. True Ferns.

This class of plants includes the well known ferns of the woods and some less frequently seen and scarcely to be recognized as ferns by the uniniated, as the water ferns and some others. The fern plant is the sporophyte generation, with roots, stems, and leaves. The latter are similar to the broad foliage leaves of seed plants, except that in most species they are divided or subdivided into leaflets, making the compound leaves so distinctive of the ferns, by which they are so easily recognized as belonging to the fern class. Some ferns have two kinds of asexual spores, and are said to be **heterosporous**; but the common ferns have only one kind of asexual spores and are therefore **homosporous.** The gametophyte is a thallus with rhizoids but without true roots.

A. **The gametophyte.** The gametophytes of many ferns, both native and those grown in greenhouses, may be obtained by sowing asexual spores on muck or peaty soil at least 8 weeks before they are wanted for study. They should be kept in a fairly warm temperature and are best watered from below rather than by spraying.

1. Examine the mature **gametophytes** growing upon soil, noting size, shape, and position. Mount one of them up side down. Make a drawing, natural size, and another sufficiently enlarged, at least one inch across, to show the location of the antheridia and archegonia with relation to the rhizoids and apical notch. Look for swimming antherozoids, sperms, and describe them and their motion if found.

B. Draw the **antheridia** and **archegonia** from fresh material,and longitudinal sections of these organs from prepared slides. What resemblances and differences do you find between these reproductive organs and those of the moss?

C. Observe a young **sporophyte growing from a gametophyte.** To what part of the gametophyte is the sporophyte attached? Notice the true roots of a young sporophyte. (A specimen will be provided for this purpose).

D. Study the fern plant, **sporophyte,** as a whole. Get the name of the species supplied. Do not break the specimens. Distinguish three parts: **rhizome** or underground **stem, roots,** and **leaf.** Make a drawing of the whole plant to show:

1. The direction of growth of the rhizome with reference to the leaf-stalk (petiole).

2. The growing apex of the stem (rhizome) or of its branches. Why is the rhizome regarded as a stem rather than as a root?

3. The old leaf-bases and their position. Do the leaves arise from the top or from the sides of the rhizome?

4. The position of the **roots.** Is their origin exogenous or endogenous? The mode of origin distinguishes roots from stems and from rhizoids.

5. The two parts of the **leaf, blade** and **petiole.** How many times is the leaf compound?

E. Study a **section of a rhizome** under the dissecting lens and microscope. Note the high degree of complexity of internal structure. Show in a diagrammatic drawing the outer layer of cells, **epidermis**; the brownish zone of thin-walled cells, **cortex**; the light-colored, thin-walled cells, fundamental **parenchyma,** of the central region; the **vascular bundles,** with large pores, the water-conducting vessels; and the very dark, thick-walled **sclerenchyma,** the fibrous masses of which give toughness with flexibility. Describe the structures observed and make a diagram of the cross section.

F. Examine the back of a spore-bearing leaf, sporophyll, of the fern, **Pteris aquilina,** the common brake or bracken, noting the reflexed margin and beneath the margin the brown sporangia. The sporangia contain asexual spores. The thin edge of the leaf covering them is a false indusium. Draw a portion of the leaf, a sporangium, and a spore. **Pteris longifolia** from the green house will serve equally well for this study.

G. Study several other species of ferns. The Boston fern, **Nephrolepis exaltata** variety **bostoniensis,** some greenhouse species of **Pteris,** or of **maiden hair ferns** may be obtained from almost any city greenhouse. Native ferns, pressed and dried, may be used also. Describe and draw a part of the sporophyll of each species, showing the characteristic form of the leaf, and the distribution of the sporangium clusters, sori, with true or false indusia whichever they may have.

ORIGIN OF NEW FORMS OF PLANTS

The Boston fern is an excellent species in which to study **new forms** that have originated suddenly under cultivation. This fern is propagated only vegetatively, as its spores do not develop normally, and are incapable of growth. **Stolons** or runners, slender leafless branches, arise near the base of the leaves and grow to a length of 6 inches or more. If pegged down to the earth these take root, produce a cluster of leaves, and thus propagate the fern vegetatively. Numerous fern-growers who grow these plants by thousands for the trade, have found among their crops of ferns a small percentage of new forms, which have arisen suddenly, "at one jump," and so different from the parent form of the Boston fern, that if the two were found growing together under natural conditions they would certainly be considered different species. Their origin being known they are called new varities, forms, or races. Within the short period of 25 years, more than **100 new varities have arisen** in this manner. Almost every florist who sells ferns at all will have one or more of these varieties for sale.

They differ from the parent form in several ways, some of which are just those differences by which we distinguish species of ferns in nature. The Boston fern has leaves that are once compound, or once pinnate, while some of the new forms have leaves that are twice or thrice pinnate. The parent form is generally a vigorous grower and the leaves attain a length of 2-3 feet. Some of the new ones are decidedly dwarf, remaining much smaller than the parent under the same conditions. Among differences found in the new varieties, are:

1. Increased division or compounding of the leaves;
2. Dwarfing in size of the whole plant;
3. Change in shape of the leaflets;
4. Crowding or compacting of the leaflets on the rachis or leaf axes;
5. Ruffling, puckering or waving of the margin of the leaflets;
6. Twisting of the leaflets.

A given variety may show one, two, or more of the above differences from the parent form. Varieties **Piersonii, Whitmanii** and others, show increased division of the leaves with normal size. **Rooseveltii** and **Teddy Jr.** show waviness or ruffling of leaflet margin, increasing from the first to the second named with decreasing size. Variety **muscosa** or the **moss-fern,** is dwarf, 6-8 inches high, with small leaflets very much crowded, twisted, and overlapping. The strikingly delicate lace fern, variety **Amerpohlii,** is very dwarf with leaves thrice-pinnate, leaflets only 3 or 4 mm. long, somewhat fan-shaped.

A. Compare several of these new varities with the typical Boston fern, noting which of the above-named characters vary from those of the parent. Show some of the differences in drawings of small portions of the plant, as well as in descriptions. Some of the new varities are not "fixed" in their characters, tending to revert to the parent form.

Among the seed plants similar phenomena take place in numerous species propagated by sexual reproduction. New forms arise in a single generation whose characters are as firmly fixed, definite, and inheritable, as are those of their parents from which they differ so strikingly. The new forms so arising are called **mutations** or **mutants.**

Considering these phenomena known among both ferns and seed plants, it seems well within the bounds of reason to say that in the production of these new varieties **we are seeing evolution go on under our direct observation.**

LYCOPODINEAE. Club-Mosses.

This class comprises one group of the **fern allies.** The common name of the class, club-mosses, indicates a superficial resemblance to the true mosses, but in reality they are more nearly like the ferns in their life history. Some of them are made into wreaths or woven into ropes or strands for decorations at Christmas.

Like the fern they have a small **thalloid gametophyte,** and a **sporophyte** with **roots, stems,** and **leaves,** with vascular bundles and complex structure. The leaves are small and look like moss leaves, but in the true moss the leafy stem belongs to the gametophyte generation, while in club-mosses it is the sporophyte generation.

In reproduction the asexual spores are commonly borne in strobili or cones, the spore-bearing leaves or sporophylls being closely crowded, in spiral or opposite arrangement, at the tips of reproductive branches.

As in the ferns, some genera have only one kind of asexual spores, which develop into gametophytes that bear male and female gametes. These are said to be **homosporous.** Other genera have two kinds of asexual spores. One is small, is called a microspore, and develops a male gametophyte bearing only antheridia and sperms. The other is much larger, is called a megaspore and develops only archegonia with eggs. This condition is called **heterospory. Selaginella** is one of the heterosporous genera.

A. **Selaginella Kraussiana** is frequently grown under the benches in greenhouses. Notice its moss-like appearance and its position in relation to the earth. The plant is the sporophyte generation. Is it upright or **repent,** that is, prostrate and rooting underneath?

1. Study a single plant with the dissecting microscope, and note the method of branching and the form and arrangement of leaves. How many kinds of leaves can you distinguish? Observe leafless branches arising from the leafy shoot and growing downwards. These are modified branches (rhizophores) which give rise to roots at their lower extremities. Draw, slightly larger than natural size, a leafy branch

showing all the parts mentioned above. Draw three times natural size a portion of a branch one centimeter long, viewed from the dorsal side, and showing the form and arrangement of leaves.

2. On a plant of **Selaginella Emmeliana** find **spore-bearing cones,** short branches upon which the slightly modified leaves, the **sporophylls,** bearing sporangia, are closely crowded together. Draw a cone three times natural size. The sporophylls making up the cones are of two kinds and bear two kinds of sporangia, viz, **megasporophylls** bearing **megasporangia** found near the base of the cone, and **microsporophylls** bearing **microsporangia,** borne in the upper part of the cone. The megasporangia are the larger and contain four large megaspores or fewer. A large number of much smaller microspores is produced in each microsporangium.

In case sections are not provided the instructor may give diagrams on the blackboard for the male and female gametophytes. In any case study the life history of Selaginella in your text book until it is clear. In its heterosporous condition it is like the seed plants, which is one of the reasons for studying it.

B. **Lycopodium.** Make a short study of a species of **Lycopodium,** noting in what respects is differs from Selaginella. Notice particularly the cones, which are larger and more prominent than those of Selaginella; but having spores and sporangia of only one kind, the species is homosporous. The gametophyte of Lycopodium is subterranean, growing under the ground.

Lycopodium powder, which may be purchased at the drug store, is used as a dusting powder, and the druggist may roll pills in it. It is composed of the asexual spores from the cones of a species of Lycopodium. Not only are these spores produced abundantly enough for such a use, but a certain grade of coal, namely, **cannel coal,** is said to be composed chiefly of fossil spores of plants similar to Lycopodium. This belief is based on the fact that microscopic sections of such coal shows a structure like the spores of that genus. Probably the spores, scattered by the wind, fell into ponds or lakes, in which they sank; and the accumulation of ages was later, under changed conditions, transformed into coal.

EQUISETINEAE. Horsetails.

A second class of the fern allies is the group **horsetails** or **scouring rushes.** The last name is given because these plants have been used as material for making scouring or scrubbing brushes. The heavy deposit of silica in their cell walls adapts them to this use, making them very resistant to abrasion.

The **gametophyte** is an irregularly-shaped green thallus. The **sporophyte** is a peculiar jointed stem with only short sheaths around the base of the internodes in place of ordinary leaves. Photosynthesis is carried on chiefly by the green stems.

A. Note the slender, usually unbranched stem of **Equisetum hyemale,** with asexual spores in cones, at the tip. **Equisetum arvense** has unbranched cone-bearing stems, and much-branched vegetative stems. This latter species is common in low wet fields, and is charged with poisoning domestic animals, even causing the death of horses. Mount spores dry and view them under low power while another person breathes gently upon them. The motion is due to the **hygroscopic action** of a pair of **elaters** on each spore. It is purely mechanical, and not at all dependent upon the action of protoplasm. The elaters of Marchantia, the peristome of the moss capsule, and the annulus of the fern sporangium, all move by hygroscopic action. In all these cases the action is concerned in the distribution of spores.

B. The fern allies were much more numerous in species, larger in size, and more important constituents of the earth's vegetation in a previous geological age, than they are at the present time, as their fossils show. The fossils also indicate that both ferns and fern allies gave rise to seed plants of types now entirely extinct. Certain plants of that ancient time had leaves like ferns, and seeds on the backs of the leaves where modern ferns have only sporangia but not seeds. Yet the fossils discovered to date do not enable us to bridge entirely the gap between modern Pteridophyta and Spermatophyta. There are many "missing links."

SPERMATOPHYTA. Seed Plants.

We have already studied the vegetative organs of seed plants, and know them to have roots, stems, and leaves of highly complex structure, with vascular bundles and numerous differentiated tissues serving for different kinds of work which plants perform. As in the ferns, the **conspicuous generation** is the **sporophyte,** and all **seed plants** are **heterosporous.** The **young pollen grain** is the **microspore,** and 4 pollen grains are formed by the **division** of a **pollen mother cell,** in which the **first division** of the **nucleus** is the **reduction division.** In all essentials therefore, pollen grains are like the asexual spores of liverworts, mosses, and ferns. The mature pollen grain produces in its interior two sperms, one of which fertilizes the egg in sexual reproduction. Hence, the pollen grain developes into a **male gametophyte,** and corresponds in particular to the **microspore** and male gametophyte of **Selaginella.**

The **megaspore** of seed plants is found in the young seeds or **ovules.** Its formation also involves a **reduction division** and it is frequently, but not invariably one of a group of 4 formed by the **division** of the same **mother cell,** three of which do not function, but disintegrate. The one functioning **megaspore,** commonly called the **embryo sac,** developes in its interior a massive, **multicellular female gametophyte** with several archegonia, as in the class **Gymnospermae,** or it develops a **female gametophyte** consisting of a small number of cells, one of which is an egg cell without a definite archegonium. This is the condition in the class **Angiospermae.**

The **egg cell,** on **fertilization** by a **sperm** from a pollen grain developes into an **embryo** or **young sporophyte,** the little plant in the seed·

The female gametophyte developes and fertilization occurs within the ovule or young seed, which is really a megasporangium with one or two integuments or seedcoats surrounding it. Since the female gametophyte is enclosed in the megasporangium, a way must be provided for the sperm to reach the egg. This is accomplished by means of a **pollen tube** which grows and **penetrates** only the **megasporangium wall** in the gymnosperms, but in the angiosperms it must not only penetrate the sporangium wall, but it must grow from the

stigma to the ovule as well, a distance which may be only a few millimeters or it may be more than a foot in some flowers.

The most striking character of the Spermatophyta, and the one which is implied in the name, is the possession of the seed as a reproductive body. As has been pointed out the **seed** is essentially a **megasporangium** having one or more enclosing envelopes, the **integuments**, which in the mature seed become the **seed coats**, and within which is enclosed a young plant together with a store of reserve food.

GYMNOSPERMAE. Naked-seeded Plants.

The seed plants of this class are nearly all woody plants of tree size and they include the **pines, spruces, firs, cedars, cycads,** and **Ginkgo,** or maidenhair tree. The two last named are the most ancient of existing seed plants.

In complexity of structure the gymnosperms are about equal to the seed plants already studied, but differ somewhat in details of structure. The large trees increase their diameters by growth of a cambium, secondary thickening and annual rings in the wood. The seeds are not enclosed in a special chamber, hence the name **Gymnospermae.** They generally lie on the free surface of scale-like structures, the sporophylls, which often become hard and woody, as in the pine cones, and protect the seeds during their development by closely overlapping until the seeds are to be scattered. Then the scales separate and the seeds fall away.

A. First make an external study of a number of gymnosperms, small branches of which may be brought into the laboratory. Identify them by means of the Key, page 99, which includes those most likely to be available for class study in Michigan, both native and introduced. The Key is based mainly on leaf characters, since most of them are **evergreens,** that is, the leaves remain on the tree throughout the year. A few of those included are deciduous, as their leaves fall in the autumn and are renewed in the spring.

Draw a portion of each species to show the distinctive characters· The instructor will explain how to use the Key. When you have identified a speciment by the Key, write its botanical and common names in your note book, and after these the characters by which you identified it in the order in which you used them in the Key. This will serve as a short, concise description.

REPRODUCTION OF THE PINE.

After identifying several pines by twigs in the laboratory, or perhaps before, you should see the same species, or some of them, as they grow out doors. You may then study their reproduction.

A. **Microsporangiate cone.** This is also called the **staminate flower** or **cone,** and the **pollen-bearing cone** or strobilus.

1. Note the **position** and **clustering** on the vegetative shoot. How many cones in a cluster? Describe the structure of one cone, noting the central axis and the **microsporophylls** or **stamens.** The parts of the sporophyll are a short stalk, two **microsporangia** or **pollen sacs,** with their supporting tissue forming the anther, and the scale-like tip. Describe the whole cone and its parts.

2. The **stamen.** Remove several stamens or **microsporophylls.** Observe them with low power in different positions to see the shape and structure. Find two elongated pollen sacs or microsporangia, full of pollen grains, or open and partly empty. Draw one stamen showing the pollen sacs and the overlapping tip of the stamen.

3. Study **pollen grains** removed from the pollen sac. Each consists of a central ovoid cell and two expansions of the outer wall, called **wings.** These are filled with air and adapt the pollen grains to distribution by wind.

4. In its early state of development the pollen grain is a **microspore,** and later, it divides up into cells which represent a **male gametophyte.** Pollen of the spruce is farther developed at time of pollination than is that of the pine, and is therefore preferable to study. Spruce pollen grains permeated with clove oil show more clearly in their interior the male gametophyte. Mount in clove oil and study this gametophyte. One

of the cells, often called the generative cell, produces two sperms. The largest cell produces the pollen tube. Draw the male gametophyte, labeling vegetative cells of gametophyte, generative cell, and tube-forming cell.

B. The **seed-bearing cone.** Study the seed-bearing cone, also called the **megasporangiate cone.** Note the very young cones, as they appear at the time of pollination. Where are they located on the twig? What is their size? Compare in both these respects with cones one and two years old. The two-year-old cone is mature and has reached its full size. Note the difference between the open and the closed cone.

1. The **woody scales** from these cones are perhaps to be regarded as megasporophylls. Draw a scale from a dissected cone showing the two ovules or seeds. These represent modified megasporangia. Note the **wing** which splits off the surface of the scale and adheres to the seed, adapting the latter to wind distribution.

2. Study a section through one of the seed-bearing scales in which the female gametophyte is in an early state of development. The ovule or young seed corresponds to a megasporangium with an outer envelope, the **integument** enclosing it. A small **opening through this integument** is called the **micropyle.** Into this micropyle the pollen grain must fall in order to complete its development, produce a pollen tube, and male gametes. Within the megasporangium the megaspore develops the female gametophyte with archegonia.

A pollen tube grows through the megasporangium wall, conveying the sperms to the egg. Draw the section, showing the integument, the micropyle, the pollen grain, a portion of the pollen tube penetrating the megasporangium wall, and the young female gametophyte.

C. If material is at hand of sections through the cone scales and young seeds of **Zamia,** one of the **Cycads,** the parts will be large enough to be seen with the naked eye, or with the small magnification of the dissecting microscope. This may, with advantage, be used in place of the corresponding part of the pine.

It should show the **megasporophyll**; the **integument**; the **micropyle,** the opening through the integument in which the pollen grain lodges at time of pollination; the **megasporangium wall** through which the pollen **tube must** grow; the **female gametophyte,** a large mass of cells that is called the endosperm in the ripe seed; and the **archegonia,** the **eggs** of which, when fertilized by a sperm from the pollen grain, develop into the **embryo,** the young plant in the seed.

All the parts should be drawn and carefully labeled and described.

D. The **Pine Seed.** Study mature seeds of white pine or other species with rather large seeds. Split the seed into halves lengthwise and study the exposed cut surfaces. On the the outside you will find a hard coat, the **seed coat** whose origin was the integument. Next a thin layer of tissue whose origin was the **megasporangium wall.** Inside of this is a large cellular mass stored with reserve food and called commonly the **endosperm.** Its origin was the female gametophyte. Lying through the center of this is the **embryo,** or young sporophyte. Its origin was the fertilized egg. It grows into the pine tree when the seed is planted. Even in the seed the embryo can be seen to have a root-end, a stem-end, and a circle of needle-shaped leaves around the apex of the stem-end. Draw a seed enlarged to twice natural size, or larger, showing all the above named parts in their relative positions.

ANGIOSPERMAE. Plants with Seeds Enclosed.

In this class of plants the ovules, or seeds, are borne in a **closed ovary,** which is the basal part of a **pistil.** At the top of the pistil a **stigma** is the organ for the reception of pollen in pollination. The **pollen tube** must grow from the stigma to the ovule. Pistils and stamens are commonly surrounded by two floral envelopes, the **calyx** and **corolla,** the four organs forming the **complete** and **perfect flowers** found in such beauty and variety among the angiosperms.

A **flowering plant, Linum, Trillium** or some other genus to be provided. The flowering plants like Selaginella and the gymnosperms are heterosporous. Their gametophytes are even more reduced than those of the latter and their sporophytes are very highly developed. The sporophyte is the plant.

A. The **mature sporophyte.** In the specimen note the stem and leaves; on the stem, nodes and internodes. Describe the plant as a whole especially the leaves, following the outline on page 10 of this manual. Make a drawing of the shoot showing stem, leaves, and flowers.

B. The **flower.** Note that the flower is made up of four kinds of structures which are believed to be the morphological equivalents of leaves. The outermost members are **sepals,** known collectively as the **calyx.** They are more leaf-like than the other members. In what ways are they like leaves? The colored structures just within the calyx are the **petals,** which are known collectively as the **corolla**; within the corolla are the **stamens,** or **microsporophylls,** and at the very center of the flower is found the **pistil,** which consists of one or more **carpels,** or **megasporophylls,** modified so as to **enclose** the megasporangia or ovules, instead of bearing them on a free surface, as in Selaginella or the pine. Draw a single flower much enlarged and show clearly the number, form and relation of all its parts. For this purpose use the dissecting microscope if necessary. Also draw a longitudinal view of the flower with calyx and corolla cut away on the side from which the flower is observed for drawing.

1. Study of single **stamen.** Observe the **filament, or stalk,** and the **anther.** Note that the latter consist of two pairs of **microsporangia,** pollen sacs, united by a tissue which is called the connective. Make an enlarged drawing of the entire stamen. Burst the anther and liberate the microspores or pollen grains. Draw a single pollen grain. It is sometimes possible to distinguish within the spore two nuclei. Their presence indicates that while still within the sporangium the microspore begins to develop into the male gametophyte.

2. Study cross-sections of the anther of the lily, genus Lilium, and draw several different stages in the development of the **pollen sacs,** or **microsporangia,** including **a mature an-**

ther with **pollen grains.** The latter are **microspores** when first formed, and **male gametophytes** when ready for pollination. In sections of the mature pollen grain note the two cells, each with its nucleus, one cell enclosed within the other. The interior one is the generative cell which forms the **male gametes.** The other forms the **pollen tube.** Draw one or more pollen grains on a large scale to show the two cells.

3. Make a large drawing of the **pistil,** noting the basal portion, the **ovary,** and the slender **style,** or **styles,** bearing at the apex the **stigma.** The stigma is a specialized surface secreting a sticky nutrient substance which retains the microspores and furnishes a suitable medium for their germination when they form the pollen tubes. Cut across the ovary with your scalpel and examine under the dissecting microscope. Make a drawing showing the ovules or megasporangia, and their place of attachment. How many chambers are there in the ovary? Do they correspond in number and position to the styles and stigmas? The number of styles and stigmas, when more than one, or **the number of chambers in the ovary,** give an indication of the number of **carpels,** or megasporophylls, which are fused to form the pistil.

4. Construct a floral diagram, following the directions given by the instructor.

C. Study the stained cross sections which will be furnished you of the ovary of a flower of the lily. These show the structure of the carpels; of the ovules, megasporangia; and of the female gametophyte, embryo sac, at some stage in its development. This gametophyte develops within the megasporangium from a single cell, the megaspore; and is even more reduced than that of Selaginella and pine. It consists normally of only eight nuclei within the embryo sac, which at the time of fertilization is composed of 7 nuclei, 2 of the 8 having fused to form one 2x nucleus. These nuclei are not usually separated by walls, though around some of them a special mass of protoplasm may be recognized, each mass of protoplasm and its nucleus constituting a cell. One such cell is the egg or ovum, which when fertilized by a sperm develops into the young plant, or sporophyte embryo, in the seed. The second sperm, or male gamete, unites with the 2x nucleus, to form a

3x nucleus, and this divides to produce nuclei that organize the endosperm of the seed. Make a drawing of the whole section and also a detail of a single ovule showing the megasporangium; integuments; nucellus, or megasporangium wall; micropyle; and the female gametophyte. Putting together your observation and the explanation of pollination and fertilization, write in your notes a brief account of the process of sexual reproduction and the seed development among angiosperms.

SEEDS AND SEEDLINGS

The flowering plants, like the pine, are characterized by the formation of the seed as the reproductive body. This is composed of a young plant or embryo, sporophyte generation, with a supply of reserve food, enclosed within one or more protective coverings, the seed coats. In some seeds the reserve food is stored within the embryo itself; in other seeds it is stored in a separate structure, the endosperm, which is found in every seed at some stage of development, but in many seeds disappears before the seed is ripe.

A. **Phaseolus vulgaris, the common kidney bean.**

1. Study a soaked bean and note its size and shape. Look for the scar, the **hilum,** where the seed was attached to its stalk in the pod, or ovary. What is the shape of the hilum? Where is it located? Find near one end of this scar a small depression, the **micropyle.** Recall that the micropyle is an opening through the integuments and was seen in the section of the ovule of the lily. The pollen tube usually enters the ovule through this opening. Draw two views of the seed, labeling all parts.

2. Remove the seed coat, noticing that the embryo completely fills the seed. Make out the following parts of the embryo or young sporophyte: a. Two large seed leaves, the **cotyledons.** In the bean the cotyledons contain the **reserve food** material. The endosperm has been destroyed and the reserve food it contained has been transferred to the cotyledons. What is their size and shape? Those flowering plants possessing two cotyledons are called **dicotyledons,** those with one cotyledon are termed **monocotyled-**

ons. b. **Hypocotyl,** the axis below the cotyledons, to which they are attached. The tip of the hypocotyl develops into the **primary root.** Indicate the location of the hypocotyl on your drawing of the whole seed. c. **Plumule,** the bud which develops into that part of the bean plant above the cotyledons. Into what organs is this differentiated? Make a drawing of the embryo three times natural size, showing all the parts.

3. Study germinating beans, noting the change in each of the structures you found in the seed. What is the function of each part? What part **first** appears and what parts finally appear above ground? What conditions are necessary to growth? Make drawings of the seedling, showing how it breaks through the surface of the soil, and the appearance when all the parts have assumed their final position.

B. **Zea Mais, Indian Corn.**

1. The grain of corn is a seed enclosed by an ovary wall. It is a ripened ovary and consequently, from a botanical standpoint, it is a **fruit.** The ovary wall and the seed coat are united so intimately as not to be readily separable. This makes it impossible to see the hilum and the micropyle. Note the size and shape of the grain, and the light-colored area where the embryo shows through the seed-coat and the ovary wall. Make a drawing from the side on which the embryo shows.

2. Split the grain longitudinally, cutting the embryo into halves from the **exposed side.** Note that in addition to the embryo an **endosperm is present.** The embryo is therefore relatively **smaller than in the bean.** Make out the following parts of the young sporophyte: a. A **single cotyledon** lying against the endosperm. This cotyledon is not a storehouse of reserve food but absorbs the food stored in the endosperm after digesting it by means of **enzymes.** Separate enzymes are necessary for the digestion of starch, oil, and protein. b. **Radicle.** c. **Plumule.** The radicle develops the root system; the plumule develops the **stem** and **leaves.** The radicle may be recognized by the fact that it is surrounded at the tip by a thick root-sheath. The plumule has a thin sheath, hardly recognizable as a separate layer.

This sheath is the first part of the plant to appear above the ground. What position do the radicle and plumule bear in relation to each other, to the cotyledon, and to the seed as a whole? Draw the section of the grain three times enlarged.

3. Make a study of the corn seedling similar to that of the bean. Do the same structures appear above the ground in both plants? What becomes of the endosperm? Of the cotyledon? Does the radicle develop into both stem and root? Draw the seedling.

C. Study some of the **common trees.** Out of doors note the tree as a whole; the shape of its top; the direction and length of its branches; the character of the bark, its color, the degree, and kind of its roughness. By these features some kinds of trees may be recognized from a considerable distance, as the American, or white elm, **Ulmus americana.** However, it is usually necessary to see the leaves of a tree, in order to recognize the species certainly, and sometimes it is also necessary to see the flowers or fruit.

Describe and draw the leaves of the species studied, following the outline on page 10 of this manual. If fruits or flowers are available, describe and draw them also.

FRUITS AND THEIR DEVELOPMENT.

Botanically a **fruit is a ripened ovary** containing the seeds, whether dry and inedible, or fleshy and edible. Commercially a fruit is the fleshy or pulpy edible part associated with the ripened ovary. This edible portion may be the whole ovary, as in a **berry.** In the drupe or stone fruit the inner part of the ovary wall becomes the stone or "pit," while the outer part is pulpy and edible. The gooseberry and tomato are examples of the berry, while plums, peaches and cherries are drupes or stone fruits.

A whole flower cluster with its reduced leaves or bracts may become thick, juicy, and edible, as in the pineapple. The whole ovary wall may be hard and stone-like, with only the seed proper edible, as in the hazelnut. The seed alone is the edible portion in the mature legume fruit, of which the bean and the pea are examples, though in the immature state the whole ovary is edible in some varieties of bean and pea.

In the material furnished you, cherry, strawberry, bean, etc., trace the development from the flower to the fruit. Dissect the flower so as to show all its parts in a longitudinal view. Make a drawing of this and also a floral diagram of the flower. Make a surface drawing and a longitudinal section of the corresponding fruit and label parts so as to show their relation to the flower. Describe the changes that take place in the different parts in developing from the flower to the fruit.

The part fruits play in the life history of the plant is best understood in relation to seed dispersal. This gives meaning to the many different forms of fruits possessed by plants.

Man grows many flowers and fruits merely for his own esthetic enjoyment, but beauty of flower and fruit are first for the benefit of the plant. Fruits attractive to animals as food are certain to have their seeds widely scattered. Many seeds pass uninjured through the digestive tract of an animal. The wide distribution of its seeds is of great importance to a plant in its success as part of the vegetation of the earth.

DOMINANCE OF ANGIOSPERMS AS LAND VEGETATION.

It is a remarkable fact that though the Angiosperms have existed upon the earth only in relatively recent geological time, (their earliest fossils are found in the cretaceous rocks), yet in that time they have been distributed over the whole land area and to some extent into fresh and salt waters, and have evolved more species than all the other classes of plants combined. Ganong estimates that 133,000 species of angiosperms are known and only 100,000 species of all the other classes of plants· If we ask ourselves how it has come about, we cannot answer the question with entire certainty; but in the present state of biological knowledge it looks as if the following factors are involved:

1. **Variations** of one kind or another produce new forms of plant life, at least some of which are better able to grow

under certain conditions than their predecessors. At present, **mutations** seem to be the kind of variations most effective in producing such new forms.

2. **Heredity** of the new characters and capacities passes them on to the offspring of the new forms, and in proportion as these multiply and are widely distributed, they are more likely to find those conditions to which they are best adapted. Numerous offspring widely distributed, with capacity to start quickly and grow rapidly are important elements in success. While seeds are not produced as numerously, and are not as easily distributed as the spores of ferns, they produce mature plants or plants of relatively large size so much more quickly than fern spores, that it is easy to understand how seed plants, when they came into existence, could compete successfully with the fern-like plants, and supplant them as the dominant vegetation. It is months or even years after fern spores are sown before mature fern plants are obtained, while many seed plants grow, flower and produce seeds and distribute them again within a few weeks. It is no accident therefore that seed plants have become the dominant vegetation of the land.

3. **Environment** and **natural selection** also play a part in evolution, but just what part has been much in controversy. Does environment produce the new forms for progressive evolution? As the evidence in favor of that view has been analyzed it has been found less convincing than it was formerly considered. It is true that plants respond to environment by producing new forms, but for the most part at least, these are just as easily changed back by reversing the environment. In other words the changes produced by environment seem not to be hereditary as are those produced by mutations. However, the question whether environment produces variations useful in progressive evolution must still be left as an open one.

It is certain however that environment frequently acts as an **eliminating** factor thus acting as a selective agency. Only such plants can grow in a desert as can make a small water supply serve through long periods of drouth. Such a plant is the cactus, a plant without leaves, with thick fleshy stems that store large quantities of water when it is available, and with a thick cuticle and deeply sunken stomata, conditions which reduce the loss of water to a minimum. There is no proof that desert conditions produced the cactus, but whether produced by mutations or by response to environment it can succeed in the desert, where the conditions eliminate all those plants which in a region of sufficient rainfall would eliminate the cactus, by overgrowing and shading it. This is an illustration of the **struggle for existence,** serving in a process of "**natural selection,**" the end result of which is that a certain group of species of plants is found in one set of conditions or **habitat** and an entirely different group of species occupies another habitat, while some species are completely eliminated, and become extinct.

Evolution, then, is believed to have come about through the interaction of **variation, heredity,** and **natural selection.** The angiosperms have become the dominant plants on land because variations in many directions have produced many species some of which are adapted to every condition of environment or habitat. The possession of seeds containing a young plant well started in the differentiation of its primary organs, root, stem, and leaf; well protected by thick seed coats and often by a hard, thick ovary wall; and well provided with a store of food with which to make a quick start and rapid growth; all these have been factors determining success in the struggle for existence in every habitat.

The most convincing evidence that evolution has been the method of creation is that to be read in the record of the rocks. The oldest known rocks contain no evidence of life. The oldest fossils to be found show that the life of that early time was of the simplest type, bacteria and blue-green algae, if the fossils have been correctly interpreted. Later, higher forms of algae appeared, and in the era known as the **carboniferous,** the age in which many of the great beds of coal were produced, **ferns, fern allies,** and **fern-like seed plants** were the dominant vegetation of the land. Still later, in **mesozoic time,** the **gymnosperms** became the dominant land plants. Only in the later period of the mesozoic time, the **cretaceous,** did **angiosperms first appear,** and in the recent, or **cenozoic time,** became the dominant land plants.

While angiosperms are found to some extent in fresh and salt water, yet the algae are now and probably have been continuously since their first appearance, the dominant plants of the water. The small size which most of them have is compensated for by their enormous numbers and rapidity of reproduction, by virtue of which there is not merely one crop a year, as is the case with wheat, corn, etc., on land; but the microscopic algae reproduce themselves, if not daily, at least over and over during the year, and thus make it possible for the sea to produce more food and support more life than an equal area of land.

If evolution has gone on in past geological time amounting possibly to hundreds of millions of years, it is probably going on in the present as rapidly as at any time in the past. It has already been pointed out that new forms are arising today, both among cultivated and wild plants, among ferns and seed plants, and the same process is taking place among lower forms of plant life also.

There are many facts of biology which have a reasonable explantion if evolution is true, but have no explanation equally satisfactory if the truth of evolution is denied. The weight of evidence is such that biologists generally have accepted evolution, not merely as a theory, but as a fact, or at least, as a working hypothesis; and investigations of the present day bearing on the problem of evolution are planned not to aid in proving the truth or falsity of the theory, but to furnish evidence as to the method by which evolution has come about. As to the method, there are different opinions held by different schools of biologists, and until they come to an agreement on the basis of evidence furnished by observation or experiment, or on reasoning from facts so learned, the proper attitude toward the problem is one of a suspended judgment, or an open mind. Scientific problems are not settled by a vote of a majority, but by the consensus of the competent. That way only, lies progress.

It is to be noted that the evolutionary point of view has been carried over to a greater or less extent into almost every field of human thought, and the modern point of view has been reached by a remarkable transformation of the thoughts of men during the sixty years that have elapsed since the appearance of Darwin's "Origin of Species."

KEY TO GYMNOSPERMS

a. Leaves broad, fan-shaped, with dichotomously branched veins, much like the enlarged leaflet of the maidenhair fern, usually 2-lobed.
Ginko biloba. The Maidenhair Tree. Hardy in southern Michigan. Often planted for ornament.

aa. Leaves pinnate, large, rigid, forming a cluster at the top of a short, thick trunk, looking somewhat like a tree fern.
Cycas revoluta. One of the **Cycads,** most of which are very similar in aspect. Common in greenhouses.

aaa. Leaves scale-like, awl-shaped, or needle-shaped.

b. Leaves scale-like, pressed closely against the twigs, which branch out in fan-like, flat sprays; fruit a small, oblong-ovoid, woody cone, 10-12.5 mm. long.
Thuja occidentalis. Arbor Vitae. White Cedar.

bb. Leaves awl-shaped or scale-like, both forms may be on one branch; fruit a fleshy berry, bluish in color when mature.
The Junipers.

1. Leaves linear-awl-shaped, in whorls of 3, 12-20 mm. long, prickly pointed, green with upper surface whitish; plant low and spreading, not of tree size, about 2-3 feet high.
Juniperus communis. The Common Juniper.
2. Leaves smaller, some awl-shaped and free at tips, some scale-like and pressed close to cylindrical twigs; a tree up to 60 feet high.
Juniperus virginiana. Red Cedar. A very valuable, reddish, odorous, durable wood.

bbb. Leaves needle-like.

c. Leaves in clusters of 2-5 on very small, dwarf branches.
The Pines.

d. Leaves 2 in a cluster.

1. Leaves 2-3.5 cm. long; cones long-ovoid, 5-7.5 cm. long.
Pinus Banksiana. Jack Pine. Gray Pine. Northern Scrub Pine.
2. Leaves 2.5-7.5 cm. long, mostly about 5 cm.; cones ovoid-conical, 3.5-6.5 cm. long; bark on upper branches reddish, flaky.